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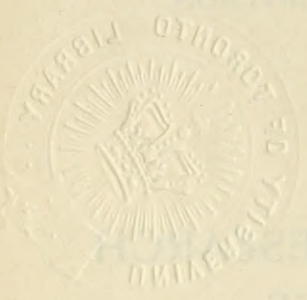
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RESEARCH INVESTIGATIONS

DICHLORAMIN-T AND PETROLATUM DRESSING FOR BURNS *

TORALD SOLLMANN, M.D.

CLEVELAND

(From The Journal A. M. A., April 5, 1919, p. 992)

Dichloramin-T as a wound antiseptic has the very real advantage of furnishing a continuous supply of the antiseptic agent, securing a continuous action over long periods of time, and this with the simplest forms of dressings. A continuous supply of antiseptic is very important in the treatment of infected tissues when it is out of the question to kill all the bacteria at once. The simplest technic is at least an important convenience.

On the other hand, dichloramin-T has some material disadvantages. The solutions must be prepared with some care, and must be fairly fresh, or else tested for the presence of available chlorin. The application causes considerable smarting and burning. This, however, disappears promptly, and can generally be tolerated. On repeated application, it is liable to irritate the skin.

These disadvantages are rather minor, in most cases. Certain physical limitations are more serious in connection with burns. The large, open surfaces require protection against mechanical irritation and access of air, and this the dichloramin-T-chlorcosane solution fails to furnish. On the contrary, this solution is absorbed by the dressings, which are then glued by

* From the pharmacologic laboratory of the Western Reserve University School of Medicine.

* This investigation was supported by a grant from the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

the wound secretions, producing pain and injury when the dressings are changed. Paraffined lace-mesh gauze does not avoid this effectively.

These drawbacks were especially conspicuous in the case of the very painful and slowly healing "mustard gas" (dichlorethylsulphid) burns that came under my observation. When these burns reach the ulcerative stage, they become so sensitive that they have to be protected by thick petrolatum dressings, especially at night. These are undesirable, since they furnish protection to the bacteria as well as to the tissues. Superficial infection therefore flourishes, and the healing must be delayed. It was attempted to compromise the matter, either by alternating the antiseptic and protective dressings or by applying a petrolatum dressing to the wound after it had been painted with dichloramin-T-chlorcosane solution (generally of 2 per cent. strength).

It was known, of course, that dichloramin-T is gradually destroyed by ordinary petrolatum, but it was hoped that the destruction would be slow enough so that some of the antiseptic would last from one dressing to the next. Subsequent chemical study of the problem showed that this expectation is not realized, and that the application of ordinary petrolatum over dichloramin-T really amounts merely to alternation of antiseptic and protective treatment.

This prompted a more detailed study of the destruction of dichloramin-T by petrolatum and various solvents. This resulted in the working out of a special petrolatum medium which was found to be sufficiently compatible with dichloramin-T for surgical purposes, so that it may be applied either mixed directly with the dichloramin-T or as a protective dressing over the dichloramin-T.

Attention may be called to the fact that liquid and semiliquid mixtures of petrolatum with active drugs are not subject to the same limitations as is the incorporation of these drugs into solid paraffin. Solid paraffin prevents adequate contact of the mass of the antiseptic with the wound. On the other hand, the

layers of liquid and semiliquid mediums in contact with the wounds are continuously changed, so that good contact is secured.

RATE OF DESTRUCTION OF DICHLORAMIN-T IN
VARIOUS SOLVENTS

The deterioration was estimated by the changes in the "available chlorin," occurring at successive periods in solutions or mixtures containing originally 2 per cent. of dichloramin-T. I am indebted to Miss J. R. Collacott for these determinations.

Estimation of "Available Chlorin."—This was carried out essentially by the method described in New and Nonofficial Remedies, 1918, p. 158. To duplicate 5 c.c. or 5 gm. samples of the mixtures to be tested, there are added 5 c.c. of glacial acetic acid, 10 c.c. of 10 per cent. potassium iodid, and sufficient carbon tetrachlorid or chloroform to thin the material (usually about 5 c.c.); then a few drops of starch test-solution, and finally, from a buret, sufficient tenth-normal sodium thiosulphate solution to discharge the color.

Each cubic centimeter of tenth-normal sodium thiosulphate solution corresponds to 0.0177 gm. of available chlorin.

The results are presented in terms of percentage of the amount of available chlorin that should have been liberated, according to the quantity of dichloramin-T originally added.

Three samples of dichloramin-T (Abbott and Squibb), two samples of chlorcosane (Abbott and Squibb) and two samples of liquid petrolatum (Squibb and Stanolind), and five samples of petrolatum (Stanolind) and one of unknown manufacture were used, with practically identical results for each instance.

Liquid Solvents.—The rate of deterioration is shown by Table 1. Carbon tetrachlorid gives the most stable solutions. Chlorcosane solutions keep practically perfect for three days, and are fairly active for a month. Liquid petrolatum solutions show some loss at once, but would preserve a fair efficiency for a month. Kerosene is surprisingly destructive, even more so than olive oil.

Petrolatum.—Commercial petrolatums are highly destructive for dichloramin-T: so much so that the efficiency is at once practically completely destroyed. This is equally true for a series of six samples repre-

TABLE 1.—TWO PER CENT. DICHLORAMIN-T IN LIQUID SOLVENTS: PERCENTAGE OF THE ADDED DICHLORAMIN T THAT REMAINS UNDECOMPOSED AT THE TIMES STATED

Solvent	Carbon Tetra- chlorid	Chlor- cosane	Liquid Petro- latum	Kero- sene	Olive Oil
No. of samples tried...	1	2	4	1	1
Period after mixing:					
At once.....	98	100	50 to 100	47	48
1 hour.....	..	86			
1 day.....	97	81 to 94	50 to 78	13	25
3 days.....	..	85 to 96		10	17
1 week.....	94	65 to 85	32 to 50	8	8
1 month.....	86	60	33	..	7
Later.....	7 weeks:79	2 mo.:34	2 mo.:29	..	4 mo.:4
		4 mo.:16 to 19			

senting different depth of colors, so that the coloring impurities are not concerned. This is shown in Table 2.

The last column contrasts this with a "paraffin ointment 3:7" prepared by mixing 30 parts of melted

TABLE 2.—TWO PER CENT. DICHLORAMIN-T IN PETROLATUM AND IN PARAFFIN OINTMENT: PERCENTAGE OF THE ADDED DICHLORAMIN-T THAT REMAINS UNDECOMPOSED AT THE TIMES STATED

Color of Petro- latum	"Superla White"	"Ivory White"	"Onyx"	"Topaz"	"Amber"	"Yel- low"	Paraffin Ointment 3:7 (2 Series)
Period after Mixing:							
At once.	15	16; 12	10	12	13	20	61-71
1 day....	10	11; 8	5	6	7	13	68
1 week...	7	8; 4	3	6	6	7	50-61
1 month	..		..	..	..	5	

surgical paraffin wax with 70 parts of liquid petrolatum (the Stanolind brands were used). There is considerable deterioration on mixing, but a practical efficiency is maintained for a week.

The physical properties of the paraffin ointment are fairly satisfactory, although it is rather more solid and damp than the commercial petrolatums.

Chlorcosane Solution Overlaid with Petrolatum, Etc.—In order to imitate somewhat the application of a petrolatum dressing over a dichloramin-T dressing, 5 c.c. of chlorcosane containing 2 per cent. of dichloramin-T were placed in bottles with 20 gm. of petrolatum, etc., without mixing. After definite periods, these mixtures were thinned with carbon tetrachlorid or chloroform, and titrated.

The results, reproduced in Table 3, again show the inferiority of petrolatum, which may destroy most of

TABLE 3.—TWO PER CENT. DICHLORAMIN-T IN CHLORCOSANE, OVERLAID WITH 20 GM. OF PETROLATUM, ETC.: -PERCENTAGE OF THE ADDED DICHLORAMIN-T THAT REMAINS UNDECOMPOSED AT THE TIMES STATED

	Liquid Petrolatum	White Petrolatum (2 Brands)	Yellow Petrolatum	Paraffin Ointment 3:7
At once.....	100	46-68	98	100
1 hour.....	95	13-62	42	99
1 day.....	101	38		
3 days.....	...	2-7	14	
1 week.....	87	3	8	60

the dichloramin-T in an hour. Both the paraffin ointment 3:7 and the liquid petrolatum were satisfactory.

CONCLUSIONS

An ointment of 3 parts of surgical paraffin and 7 parts of liquid petrolatum has relatively little destructive action on dichloramin-T and can be used as a protective dressing on wounds (burns) treated with dichloramin-T-chlorcosane solution, and even as a basis for a dichloramin-T ointment.

Ordinary petrolatum, irrespective of its color, is very destructive of dichloramin-T, and cannot be used effectively in connection with it.

Liquid petrolatum can be used in emergencies as a vehicle for dichloramin-T, although it is inferior to chlorcosane.

Solutions of dichloramin-T in carbon tetrachlorid are very stable, while those in kerosene or in olive oil deteriorate very rapidly.

IODIN TINCTURES, WATER SOLUBLE *

TORALD SOLLMANN, M.D.¹

(From *Journal of the American Pharmaceutical Association*, July, 1919, p. 534)

Water-soluble tincture of iodin, devoid of potassium or sodium iodid, have been widely advertised as superior to the U. S. P. tincture, under the claim that potassium iodid "produces a localized irritation." It is, of course, highly improbable that potassium iodid should be more irritant than the hydriodic acid that is present in these preparations; in fact, a substance would need to be a fairly powerful irritant to modify the irritation of the free iodin to a noticeable degree. However, it appeared advisable to subject the claim to direct tests, using the secret "Burnham's Iodin" and "Surgodin," and comparing them with analogous non-secret preparations, as well as with the official tinctures. The results of the physiologic tests, which are reported elsewhere, confirmed that the claims of superiority are untenable. However, the formulas that were devised for the nonsecret preparations may have some pharmaceutical interest.

Effect of Hydriodic Acid on the Solubility of Iodin.
—Preliminary assays of Burnham's iodin and of surgodin showed that they contain from 2.2 to 3.2 per cent. of free iodin, and a variable amount of combined iodin in a volatile form, chiefly hydriodic acid (1.2 to 2.6 per cent.). It is quite probable that the hydriodic acid is not added as such, but is formed in secret processes of preparing these mixtures. In any case, however, it seemed probable that it represents the solvent agent, and it was therefore planned to add it directly to the alcoholic iodin solutions.

* This investigation was supported by a grant from the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

1. From the Department of Pharmacology of the Medical School of Western Reserve University, Cleveland.

The specimen of concentrated hydriodic acid was obtained from the Central Scientific Company, Chicago, and marked Specific Gravity 1.70. It assayed 0.95 gm. of HI per c.c.

Increasing quantities of this acid were added to 7 per cent. and to 3 per cent. alcoholic solutions of iodine, until the mixtures could be poured into water without turbidity. One hundred c.c. of the 7 per cent. iodine required 1 c.c. of the (HI specific gravity 1.7); 100 c.c. of 3 per cent. iodine required 0.1 c.c. of the HI (specific gravity 1.7).

It is noteworthy that the more dilute solutions of iodine require an even smaller ratio of hydriodic acid. Furthermore, the 7 per cent. solution, when made up with the minimum of HI, is not miscible in all proportions. It is therefore safer to increase the hydriodic acid.

Tincture Iodi Hydriodica	7 per Cent.	3 per Cent.
Iodin.....	7 gm.	3 gm.
Hydriodic acid, sp. gr. 1.7.....	2.2 c.c.	1 c.c.
Distilled water.....	5 c.c.	
Alcohol, q. s.	100 c.c.	100 c.c.

I therefore used the two following formulas, which I found miscible with water in all proportions tried; in fact, forming clearer solutions than Burnham's or surgodin:

The 3 per cent. solution is practically the equivalent of the secret products and seems to be identical with surgodin.

Other Solvents.—A few attempts were made with other substances that suggested themselves during the course of the work. They were unsuccessful, but may be briefly recorded.

Ethyl Iodid.—This is a good solvent for iodine, but is insoluble in water. For instance, a mixture of one volume of 3 per cent. alcoholic iodine solution and two volumes of ethyl iodide, when poured into water, separates into two layers, of which the watery layer is colorless.

Glycerin.—This mixes freely with 7 per cent. alcoholic iodine, but the iodine precipitates on the addition of water, even when large quantities of glycerin are used.

Lactic Acid.—Concentrated lactic acid mixes freely with 7 per cent. alcoholic iodine, but precipitates on dilution with water. A fairly high concentration of the acid is required to maintain solution.

Analytic Results.—The various preparations were examined for *free iodine* by titration with thiosulphate by the U. S. P. method, using 5 c.c. of the 7 per cent. preparations and 10 c.c. of the 3 per cent. solutions. The *acidity* was determined in this with N/10 NaOH. The *total iodine* was liberated by nitrite and acid, extracted with chloroform, and titrated with thiosulphate.

The assays were carried out by Miss J. R. Collacott. The results are shown in Table 1.

TABLE 1.—ANALYSES OF IODINE PREPARATIONS
(GM. PER 100 C.C.)

Nature of the Preparation	Free Iodine	Acidity as HI	Total Iodine	Combined Iodine*
Burnham's soluble iodine.....	2.2-2.8	1.4	5.5	2.7
Surgodine.....	3.0	1.2	4.7	1.7
7% hydriodic tincture.....	5.7	2.1	7.8	2.1
Iodine..... 7 gm.				
Hydriodic acid (1.7)..... 2.2 c.c.				
Distilled water..... 5 c.c.				
Alcohol q. s. 100 c.c.				
3% hydriodic tincture.....	3.1-3.2	0.8-1.0	4.0-4.2	0.8-1.1
Iodine..... 3 gm.				
Hydriodic acid (1.7)..... 1 c.c.				
Alcohol q. s. 100 c.c.				

* Inclusive of HI.

CONCLUSIONS

The paper presents formulas for water-miscible *hydriodic tinctures of iodine*, containing 7 per cent. and 3 per cent. of free iodine.

These are comparable to the secret "soluble iodines."

None of these, however, present any material therapeutic advantage over the U. S. P. tincture, or the simple alcoholic iodine solutions, in their respective fields.

COMPARATIVE ACTIVITY OF LOCAL ANESTHETICS

VI. DIFFICULTLY SOLUBLE ANESTHETICS ON MUCOUS MEMBRANES *

TORALD SOLLMANN, M.D.

CLEVELAND

(From *The Journal of Pharmacology and Experimental Therapeutics*, August, 1919, p. 429)

METHODS

The efficiency of the difficultly soluble anesthetics for mucous membranes may be estimated, although somewhat roughly, on the cornea of rabbits, or on the gums of human subjects. In either case, the threshold values are compared by using dilutions made with powdered talcum. Neither method is altogether satisfactory, but they check each other quite well, and should show marked differences in efficiency, if such exist.

CORNEAL METHOD

In making the tests, the rabbits are placed in snugly fitting individual stalls. The lashes are clipped. The winking reflex is tested by touching the cornea with a pointed pencil. The anesthetic, diluted to the desired degree with talcum, is then applied with a simple powder blower. This consists of a glass cannula attached to a rubber syringe bulb. The tip of the cannula is filled by pressing it into the anesthetic powder, and this is then blown on the cornea. The sensitivity is tested after five minutes, repeating the applications until the cornea can be touched without winking, or, if the powder is ineffective, until six appli-

* The investigation was supported by a grant from the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

* From the Pharmacological Laboratory of the School of Medicine of Western Reserve University, Cleveland.

* Received for publication May 19, 1919.

cations have been made. If anesthesia occurs, the observations are continued, at five minute intervals, until sensation returns.

RESULTS OF THE CORNEAL METHOD

These are shown in Table 1. I am indebted to Miss J. R. Collacott for the observations. A indicates the number of applications (with five-minute intervals) required to produce anesthesia, "nil" meaning that anesthesia did not result after six applications. R signifies the minutes elapsing between the last application, and complete recovery of sensation. In the

TABLE 1.—INSOLUBLE ANESTHETICS ON RABBITS' CORNEA
THE SYMBOLS ARE EXPLAINED IN THE TEXT

Per- centage	Anesthesin		Cycloform		Orthoform-New		Propoesin	
	A	R	A	R	A	R	A	R
100.0					3; 3	25; 20		
75.0					3	25		
50.0	3	15	4	15	4	30	2	30
35.0					3	20		
25.0	3	25	5	10	3	20	3	20
10.0	3; 4*	15; 10	5; 3*	10; 15	2	15	3	15
5.0	4*; nil	10	3*; nil	10	nil; nil		5; nil	5
2.5	nil	nil			nil; nil		5; nil	5

observations marked *, the nature and percentage of the anesthetic were unknown to the operator.

APPLICATION TO THE GUMS

The powder is applied with a spatula to the outside of the gums, and well into the sulcus of the lip. The observations were made on one subject, at the left second incisor. Usually only one substance was tried each day. The sensibility was tested with a pointed pencil. The applications were renewed every two or three minutes, until the maximum effect had been reached, or until some six applications had proved ineffective. The inaccuracies of the method arise mainly from the rather poor sensitiveness of the normal gums.

RESULTS OF APPLICATIONS TO THE GUMS

These are shown in Table 2.

TABLE 2.—ANESTHESIA FROM INSOLUBLE ANESTHETICS ON THE GUMS

Percentage	Anesthesin	Cycloform	Orthoform-New	Propaesin
25.0		Almost complete (6)	Almost complete (2)	
10.0	Almost complete (4)	Slight (5); almost complete (3)	None (6); doubtful	Almost complete (4)
5.0	Almost complete (4); doubtful (5)	Almost complete (2)	Slight (4)	Considerable (3)
2.5	Very slight (6)	Almost complete (3)	Doubtful (4)	Slight (4)

The numbers in parentheses are the numbers of applications required to produce the maximal effect.

CONCLUSIONS

The efficiency of the difficultly soluble local anesthetics for mucous membranes may be demonstrated on the cornea of rabbits, or on the gums of human subjects. An approximate estimate of their relative efficiency may be obtained by comparing dilutions made with talcum powder.

On the cornea, anesthesin, cycloform, orthoform-new and propaesin appear to be practically equivalent.

They are certainly effective in concentrations of 10 per cent.; uncertain at 5 per cent., and practically ineffective at 2.5 per cent.

The anesthesia persists for from ten to fifteen minutes with 10 per cent.; fifteen to thirty minutes with 25 to 100 per cent.

On the gums, a greater variation was noted. The minimum concentrations giving almost complete anesthesia were as follows:

	Per Cent.
Cycloform less than.....	2.5
Anesthesin.....	2.5 to 5.0
Propaesin.....	5.0 to 10.0
Orthoform-new.....	10.0 to 25.0

A FURTHER CONTRIBUTION TO THE PHARMACOLOGY OF THE LOCAL ANESTHETICS *

CARY EGGLESTON

AND

ROBERT A. HATCHER

NEW YORK

*(From The Journal of Pharmacology and Experimental Therapeutics,
August, 1919, p. 433)*

The search for substitutes for cocain has led to the introduction into the materia medica of a large number of compounds possessing the property of causing local anesthesia, and for each of these the claim has been made that it is less toxic than cocain. These compounds belong to several general chemical groups. As we shall show later, not only do these compounds of somewhat diverse chemical nature possess the common property of producing local anesthesia, but also they resemble one another very closely in all their more important pharmacologic actions, such differences as are shown being chiefly quantitative. That they are all capable of producing severe, or even fatal, acute poisoning in man, with symptoms closely analogous to those produced by cocain, will also be seen in the brief review of the more important literature which we give before passing to the presentation of our experiments.

REVIEW OF THE LITERATURE

The occurrence and symptoms of acute cocain poisoning are so well known that it is unnecessary to do more than refer to one or two articles. Mattison ¹

* From the Department of Pharmacology, Cornell University Medical College, New York City.

* Received for publication, June 3, 1919.

* Part of the expense of this research has been defrayed by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

1. Mattison, J. B.: Med. and Surg. Rep., **65**: 645, 1891.

presents in abstract a large number of acute fatal and nonfatal cases of cocain poisoning; and a good bibliography of cocain poisoning is to be found in a paper by Wildenrath.² In a previous paper we³ cited the fact that death has been recorded following such small doses of cocain as 16, 40 and 60 mg., respectively, while 1.25 gm., injected subcutaneously, have been survived. The typical symptoms and course of acute, nonfatal intoxication are described by Lodge.⁴ The patient, a man 40 years old and in good health, received a subcutaneous injection of 20 minims of a 2 per cent. solution of cocain—about 13 mg. of the drug. In from two to three minutes the patient became nervous with heightened reflexes, rapid pulse, deepened respiration, vertigo and flickering before his eyes. Nausea developed in about ten minutes, followed by vomiting. At the end of about an hour after the injection the toxic symptoms were at their height, when the following were noted:

Pulse 120, respirations deepened, the inspiratory time probably increased to four times the length of the expiratory time; reflexes greatly increased, clonic spasms of all the muscles of the limbs, arms and inferior maxillary . . . pupils markedly dilated . . . muscles of the lower jaw were in tonic contraction.

The patient recovered in about two hours after the injection, but he did not sleep well that night, and had a severe headache the next day. This case illustrates the promptness of beginning absorption and the rather prolonged action of the cocain—both facts of importance, as will be shown subsequently.

The literature of tropacocain is comparatively meager, and we have not found any record of fatal intoxication in man from the use of this drug. We venture to express it as our belief, however, that this

2. Wildenrath, Robert: *Friedreich's Blätt. f. gericht. Med.* **62**: 215, 1911.

3. Hatcher, Robert A., and Eggleston, Cary: *Jour. Pharm. and Exp. Therap.* **8**: 385, 1916.

4. Lodge, E. Ballard: *Dental Summary*, **32**: 44, 1912.

drug is little, if any, less toxic for man than is cocain, though it is possible that man may not show the same idiosyncrasy toward it as he does toward cocain.

Marcinowski,⁵ in an extensive paper on the clinical uses of beta-eucain, comments on its low toxicity for man. He says that cocain is 3.75 times as toxic for animals as beta-eucain, but he does not mention the species, the mode of administration, or the concentration of the solutions, hence his figures are virtually worthless. He gives a bibliography of 202 papers, chiefly clinical, and he goes so far as to state that where beta-eucain can be substituted for cocain it is little short of criminal negligence not to do so. He also makes the direct statement that in man general intoxication has been seen only after the use of beta-eucain in the spinal canal.

Kraus⁶ records a case of severe acute poisoning from the injection into the urethra of a healthy man of 0.2 gram of beta-eucain in 2 per cent. solution. The day preceding, the same dose had been similarly injected for the incision of a stricture, and had produced no symptoms whatever. He believes that the acute intoxication resulted from the rapid absorption of the drug through the recent wound.

Way⁷ also records a case of serious intoxication from the infiltration about the genitals for circumcision of 0.12 gram of beta-eucain in a solution containing a few drops of epinephrin. In both this and the preceding case the symptoms appeared promptly after the injection, and in both clonic or tetanic convulsions were noted. Recovery was apparently more rapid in Way's patient than is seen after cocain, while Kraus' patient still had some symptoms after the lapse of nearly two hours.

The more important literature of intoxication by stovain has been reviewed in a paper by Smith and Hatcher,⁸ to which the reader is referred. It is well

5. Marcinowski: *Deutsch. Ztschr. f. Chir.* **65**: 417, 1902.

6. Kraus, Joseph: *Deutsch. med. Wchnschr.* **32**: 67, 1906.

7. Way, L. F. K.: *Jour. Roy. Army Med. Corps* **23**: 209, 1914.

8. Smith, Maurice I., and Hatcher, Robert A.: *Jour. Pharm. and Exp. Therap.* **9**: 231, 1917.

known that many cases of serious, or fatal, intoxication have resulted from the use of this drug, especially for intraspinal anesthesia.

Alypin was introduced into the materia medica by Impens and Hofmann with claims of superiority over cocain and the cocain substitutes, and Impens⁹ presents an extensive pharmacologic study of its actions and toxicity, in which he states that for cats and dogs alypin is only half as toxic as cocain by subcutaneous injection. He concludes from his experiments, that the danger from alypin in man must also be much less than that of cocain. He, however, shows clearly that the toxic actions of alypin and cocain are essentially the same qualitatively in the lower animals, although he contends that there is less tendency to variations in the responses of different animals to a given dose of alypin than to cocain. *

That severe intoxication does occur with alypin is seen by the following references to recorded cases. Garasch¹⁰ records two cases of severe poisoning resulting from the intra-urethral instillation of 5 c.c. of a 5 per cent. solution, and 5 c.c. of a 2 per cent. solution, respectively (0.25 and 0.1 gm., respectively), in two young men for the purpose of instrumentation. The symptoms began in from one and a half to two minutes, rapidly reached their maximum, and both patients had fairly completely recovered within about twenty minutes. In both instances the heart and respiration had stopped, or nearly stopped, and convulsions were repeated. The author says that these are the only cases of severe intoxication from alypin in his total of 1,453 cases of its use in genito-urinary surgery. He also records the injection into the bladder of an old man with carcinoma of that viscus of 15 c.c. of a 10 per cent. solution (1.5 gm.) of alypin without the production of any symptoms.¹¹

9. Impens, E.: *Arch. f. d. ges. Physiol.* **110**: 21, 1905.

10. Garasch, B. A.: *Russky Vratsch* **10**: 1281, 1911; also abstract in *Zentralbl. f. Chir.*, **38**: 1438, 1911.

11. Translated by Mr. Solomon Krell, technical assistant.

Ritter¹² records an instance of acute intoxication resulting in death, following the infiltration into the region of the thyroid gland of about 1 gm. of alypin in 2 per cent. solution. Although the initial symptoms were typical of acute alypin poisoning, it is not certain that death was wholly due to that drug, as the patient was suffering from obstructed breathing due to a goiter, and had also received morphin and adalin before the infiltration.

Other cases of severe or fatal intoxication have been reported; thus Proskauer¹³ records sudden death after injection into the urethra of 20 c.c. of 2 per cent. solution of alypin (0.4 gm.) following injury to the mucosa by attempted passage of a cystoscope; and Schroeder¹⁴ records two nonfatal cases following intranasal infiltration of 13 c.c. and 18 c.c., respectively, of a 2 per cent. solution of alypin (0.26 and 0.36 gm.), followed by application by spray and tampons of a 15 per cent. solution in small amounts. Both of the patients had previously received 0.6 mg. of scopolamin. In both patients the symptoms of intoxication began in less than ten minutes after the injection of the alypin, and both recovered promptly after artificial respiration and the administration of cardiac stimulants. Jacobs¹⁵ records two nonfatal cases following the intra-urethral instillation of 2 per cent. alypin solution, both patients having sustained injury to the urethra by previous effort to pass an instrument. Each of these patients received a dose of about 0.16 gm. of alypin, and although both had violent clonic convulsions and stopped breathing, recovery was very rapid following artificial respiration.

We have not found any references to severe acute poisoning by either holocain or nirvanin, but this is probably due to the fact that neither of these drugs has been extensively employed in clinical practice. Certainly it is not due to their slighter toxicity.

12. Ritter, G.: *Med. Klin.* **8**: 1236, 1912.

13. Proskauer, Arthur: *Therap. d. Gegenwart*, **54**: 555, 1913.

14. Schroeder: *Deutsch. med. Wchnschr.* **39**: 1459, 1913.

15. Jacobs, Louis Clive: *Cal. State Jour. Med.* **15**: 268, 1917.

The question of accidental poisoning by procain (novocain) has been reviewed in our earlier paper and does not require further mention, except that we wish to emphasize the fact that in the nonfatal cases recovery from the threatening symptoms has generally been rapid. Following the publication of that paper a plea for the reporting of all cases of accidents from the use of local anesthetics was published broadcast in the medical, dental and pharmaceutical journals, signed by Torald Sollmann, chairman of the Therapeutic Research Committee of the Council on Pharmacy and Chemistry, and by Robert A. Hatcher. Although more than a year has elapsed since that plea must have reached every physician, dentist and pharmacist who reads his journals, only four cases of procain and five of cocain poisoning have come to our attention, all privately reported. In but two of these cases were the details given sufficient to warrant their mention. One was a case of severe intoxication in a woman 30 years old and on the verge of a nervous breakdown. Forty minims of 2 per cent. solution of procain (about 50 mg.), containing 1:50,000 epinephrin, were injected into the mandibular foramen and less than eight minutes later the patient developed clonic convulsions and slowing of the pulse. Camphorated oil and aromatic spirit of ammonia were administered and recovery was complete in less than two hours. The second case resulted from the massage into the nose of about 65 mg. of cocain in a solution containing 10 drops of 1:1,000 epinephrin. The patient promptly had three seizures of clonic convulsions with great weakening of the heart but recovered in half an hour, aromatic spirit of ammonia having been employed as a stimulant.

Two facts seem to stand out clearly from the preceding review:

1. That moderate doses of cocain and its substitutes are capable of producing severe or fatal acute intoxication in man under a variety of conditions, both with respect to the patient and to the mode of application of the drug.

2. That the rapidity of absorption of the drug into the blood stream seems to be the factor of greatest importance in determining the occurrence of acute intoxication, especially in the case of the several substitutes for cocaine.

It will be admitted by all that it would be of the greatest value to determine accurately the several factors which lead to the production of such acute intoxications; their relative importance; their relative frequency of occurrence, and the methods of anticipating them or of avoiding them. But, while animal experiments may throw some light on these problems, their accurate solution can come only from the collection and careful study of clinical accidents. It is, therefore, of first importance that every one who encounters a case of accidental intoxication should report his observations with as great accuracy and detail as he can. It is no reflection on the physician or dentist that he should occasionally meet with accidental poisoning from these drugs, but rather is it a reflection that he should be unwilling to publish his own experiences in their fullest detail and aid in the future avoidance of similar accidents.

EXPERIMENTAL

The present investigation was undertaken in the attempt to throw some light on the causes of acute intoxication in man produced by the several local anesthetics; to discover, if possible, some of the factors which influence the toxicity of these drugs; and to find means of avoiding or combating their acute toxic actions. We have therefore made observations on the following points:

1. The phenomena of acute intoxication after intravenous injection.
2. The maximum toxicity by rapid intravenous injection.
3. The mutual synergism of the several local anesthetics.
4. The toxicity by slow or repeated intravenous injections.
5. The toxicity by subcutaneous injection.
6. The influence of epinephrin on toxicity by subcutaneous injection.
7. Notes on absorption from mucous membranes.
8. Elimination by the liver.
9. Efforts to influence the toxicity of the local anesthetics.
10. Synergism with epinephrin on systemic administration.
11. Measures for resuscitation.
12. Antagonism by ouabain.

More than 300 experiments were performed; hence we will present only a few typical protocols and give the general results in the form of tables and discussions. We have also drawn on the results of previous work in our laboratory. All of the experiments were made on cats, which more closely resemble man in their responses to most drugs than do the rodents, especially with reference to absorption from the subcutaneous tissues. Dogs were not used because, owing to their larger size, we would not have had sufficient supplies of most of the drugs to carry out the investigations planned, and further supplies were unobtainable; and also because we had previously shown that these animals behave essentially as do cats to the local anesthetics. We omitted the use of rabbits, in part because of their almost prohibitive expense, and because it has been shown that they do not differ essentially from cats in their susceptibility to these drugs by rapid intravenous injection (Schroeder, Hatcher and Eggleston, Smith and Hatcher).

The following local anesthetics were employed, the hydrochlorids being used throughout:¹⁶ alypin, apothecin, beta-eucain, cocain, holocain, nirvanin, procain (novocain), stovain, tropacocain.

Except where otherwise indicated, all operative procedures were carried out without the use of a general anesthetic. Where it was necessary to expose a vein or an artery the hair was clipped closely and a 10 per cent. solution of phenol in oil was used as a local anesthetic. In all experiments, except where stated to the contrary, the local anesthetic was dissolved in 0.85 per cent. sodium chlorid solution. In every instance the solutions were prepared on the day on which they were to be used, except in the case of tropacocain, which was sometimes kept in solution for from two to three days.

16. We are indebted to Prof. Torald Sollmann for having generously provided us with supplies of several of the synthetic cocain substitutes at a time when these were not commercially available.

1. PHENOMENA OF ACUTE INTOXICATION AFTER INTRAVENOUS INJECTION

When a severely toxic, but not fatal, dose is rapidly injected into the femoral vein the general symptoms produced are essentially the same for all of the local anesthetics studied. Within a few seconds there are more or less pronounced stimulation of the respiration and an abrupt fall of blood pressure, the heart rate

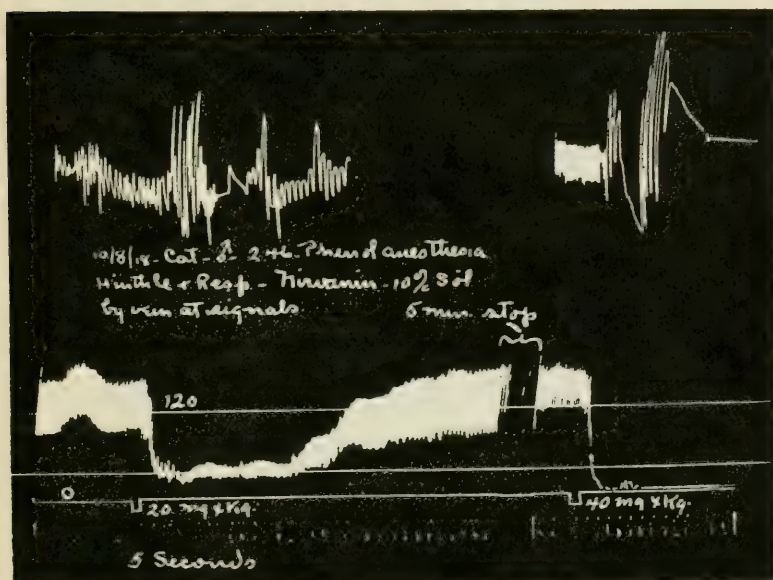


Fig. 1.

varying, but usually being slowed. The respiration may then stop for several seconds, or may continue to show stimulation, both rate and depth being increased. Within about a minute the heart begins to improve and the blood pressure rises; at about the same time the respiratory stimulation begins to pass off, and commonly at about this time there is one of several brief but severe clonic convulsions. Within less than five

minutes the animal is apparently quite normal. The fall in blood pressure is due to a weakening of the heart and not to any immediate vasodilatation. The length of time required for the onset of symptoms does not differ materially for the different drugs or for doses of different size, so long as the dose is a toxic one. There is, however, some difference noted for each of these factors in the rate of recovery. After doses just sufficient to produce the phenomena described complete recovery seldom takes over five minutes, but when the doses are nearly fatal the time of recovery may be about doubled, repeated clonic convulsions being continued for from six to ten minutes after the injection. The different drugs, also, may be divided into two groups with reference to the rate of recovery following a single sublethal dose. Cocain and holocain are the only members of the first group, and are characterized by a decidedly more prolonged action than is seen after any of the other cocain substitutes. This difference will be more clearly shown when we come to the discussion of repeated doses, and it is one of prime importance. The second group comprises all the other drugs studied; which show only slight differences in the rates of recovery, and from all of which recovery is rapid.

The effects on the heart and circulation, on the respiration, and the rapid recovery from these effects, are shown in the tracing—Figure 1—which accompanies the following protocol.

Protocol 1. Cat, male, 2.46 kg.

Twenty milligrams $\times$ kg. nirvanin in 10 per cent. solution at once into femoral vein. Sharp fall of blood pressure in less than ten seconds, followed at once by respiratory stimulation. Then respiration almost stopped, while heart began to show slight recovery. Within two minutes after the injection the blood pressure had returned to normal. Clonic convulsions interfered with the respiratory tracing.

Recovery was complete in less than ten minutes; when a surely fatal dose was given and followed by prompt cessation of the heart, transient respiratory stimulation and death.

When fatal doses are given there is often an almost instantaneous general extensor spasm, combined with fixation of the respiration and sudden cessation of the heart beat. After an interval of less than thirty seconds the respiration usually returns in the form of a few shallow gasps, while the heart may resume beating feebly and slowly and continue for a few seconds to several minutes. At other times the auricles alone continue to beat for several minutes, the ventricles not participating, as shown by arterial tracings or by direct

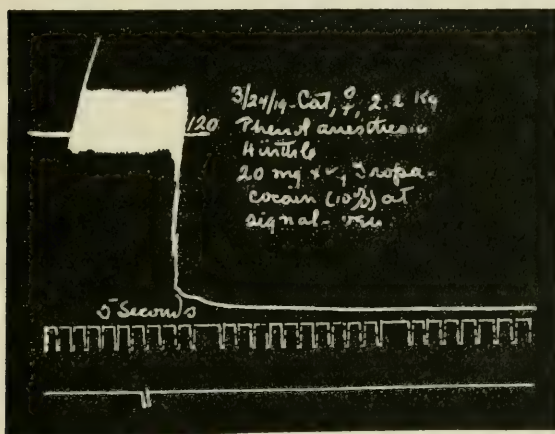


Fig. 2.

inspection of the heart. The abruptness with which the heart may be brought to a standstill is shown in the tracing—Figure 2—accompanying the following protocol.

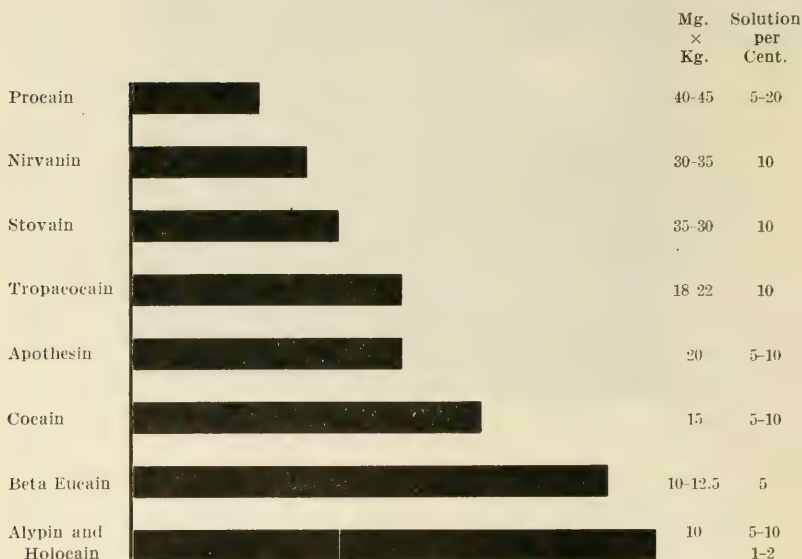
Protocol 2. Cat, female, 2.2 kg.

Twenty milligrams $\times$ kg. tropacocain in 10 per cent. solution at once into femoral vein. Almost instantaneous, violent extensor spasm, respiration fixed; heart stopped suddenly in ten seconds. Few respiratory gasps. Death.

The outstanding features of the phenomena of the toxic actions of all the local anesthetics, when injected rapidly into the circulation are:

1. The rapidity of onset of the effects on the heart and respiration.
2. The rapidity of complete recovery, even after doses which are sufficient to stop both the heart and respiration, which is true of all of the drugs studied, except cocaine, with which the recovery is decidedly slower.

CHART 1.—SHOWING RELATIVE TOXICITY: FATAL DOSES IN MILLIGRAMS PER KILOGRAM; AND RANGE OF CONCENTRATION OF SOLUTIONS FOR THE SEVERAL LOCAL ANESTHETICS



2. MAXIMUM TOXICITY BY RAPID INTRAVENOUS INJECTION

The toxic actions of the local anesthetics being exerted only after the entrance of the drugs into the circulation, we sought to determine the approximate maximum toxicity of each after very rapid intravenous

injection of concentrated solutions. We found that it made little difference whether the solution employed was 5 per cent. or 20 per cent.: since within those limits the dose could be injected from a syringe in from five to fifteen seconds. For the sake of accuracy, however, we append the concentration employed for

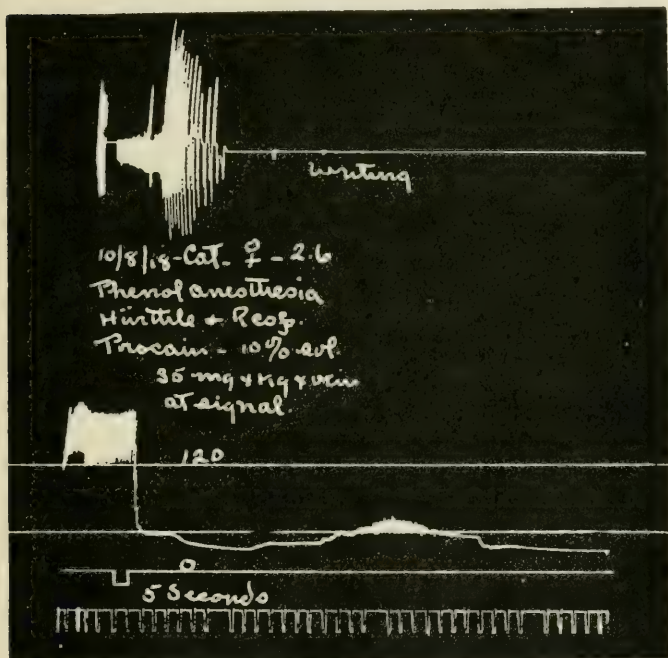


Fig. 3.

each of the drugs. (Chart 1 presents in graphic form the relative toxicities of the nine compounds studied; gives the minimal fatal dose in milligrams per kilogram; and shows the range of strengths of the solutions employed. We have taken our figures for procain and stovain in part from our previous papers.

In all cases death is apparently due to the essentially simultaneous paralysis of the heart and respiratory center. In some cases the heart may return and beat feebly for a few seconds after the respiration has failed (see Fig. 3), while in others there may be some furtive efforts at respiration after the heart has stopped. The direct action on the heart is shown in a tracing taken from a perfused heart by Smith and Hatcher, and in the immediate stoppage of the heart recorded in some of the tracings we reproduce here.

From the chart it is seen that cocain occupies an intermediate position with reference to its maximal toxicity, being surpassed by alypin, beta-eucain and holocain, while the least toxic are nirvanin and procain. It is interesting to note that alypin, which is chemically closely related to stovain, is about three times as toxic, while cocain and tropacocain, which are very closely related, differ appreciably in toxicity, cocain being a third more toxic than tropacocain.

3. MUTUAL SYNERGISM OF THE SEVERAL LOCAL ANESTHETICS

The striking similarity noted between the systemic actions of all of these local anesthetics, especially on the heart and respiratory center, led us to conduct a series of experiments to determine whether or not they are synergistic, and if so to what extent. The accompanying protocols illustrate our methods of procedure and the results obtained.

Protocol 3. Cat, male, 3.46 kg.

Twelve milligrams $\times$ kg. nirvanin (37 per cent. of fatal dose) plus 18 mg. $\times$ kg. procain (43 per cent. of fatal dose) injected at once into femoral vein in 10 per cent. solutions.

Immediate violent respiratory stimulation and distress; heart did not stop; typical rapid recovery.

The total represented 80 per cent. of the combined fatal doses.

Protocol 4. Cat, female, 2.2 kg.

Five milligrams $\times$ kg. alypin (50 per cent. of fatal dose) plus 7.5 mg. $\times$ kg. cocain (50 per cent. of fatal dose) at once into femoral vein, in 10 per cent. solutions.

Immediate respiratory stimulation; heart stopped in about ten seconds; respiration stopped in about one and a half minutes.

The total represented 100 per cent. of the combined fatal doses.

These two protocols are typical of our results throughout, which showed that all of the local anesthetics studied are mutually and quantitatively synergistic. Thus the administration intravenously of a mixture of any two is followed by the general train of symptoms already described, or the administration of a measured fraction of the fatal dose of one plus such a fraction of any other as will make the two fractions equal to 100 per cent. of the combined fatal doses of the two results in death with symptoms which are typical of the whole group. Nothing would be gained by the further multiplication of protocols.

4. TOXICITY BY SLOW OR REPEATED INTRAVENOUS INJECTIONS

We have emphasized, in the section dealing with the maximum toxicity of the local anesthetics by intravenous injection, the fact that the fatal dose is thrown into the blood stream very rapidly—in fact virtually all at once. We have also mentioned the fact that recovery from a sublethal dose is very rapid, except in the case of cocain, even when the dose approaches closely to the fatal. We have shown in our earlier paper that many times the minimal fatal dose of procain (then called novocain) can be injected intravenously if the injection be made slowly, or if a series of sublethal doses be given at intervals of about fifteen minutes. The same was also shown to be true of stovain by Smith and Hatcher.

In our paper on procain we gave a protocol showing that cocain could not be given by repeated intravenous injection of small doses without the development of evidences of cumulation, as shown by the increasing severity of the symptoms following the latter repetitions, and by the ultimate death of the animal after a total of five times the minimum single fatal dose, given

in a period of four hours and sixteen minutes in a series of fifteen injections. We have confirmed this observation and have investigated the remaining drugs in this series in the same way, as well as by slow and nearly continuous injection of relatively dilute solutions. Before discussing the results of this portion of our work we will present two abbreviated protocols.

Protocol 5. Cat, female, 2.46 kg.

10:17 a. m.: 20 mg. $\times$ kg. nirvanin at once into femoral vein, in 5 per cent. solution. Almost immediate convulsion; heart impalpable; enormous respiratory stimulation; followed by prompt recovery.

10:32 a. m.: 20 mg. $\times$ kg. nirvanin as above; symptoms as before, but heart did not stop.

10:47 a. m.: 25 mg. $\times$ kg. nirvanin as above; symptoms as before.

11:07 a. m.: 30 mg. $\times$ kg. nirvanin as above; symptoms as before.

11:30 a. m.: 40 mg. $\times$ kg. nirvanin as above; symptoms as before; but heart stopped almost instantly. After half a minute heart began to beat again.

11:32 a. m.: Animal nearly normal.

Recovery complete.

This animal received 135 mg. $\times$ kg. of nirvanin—more than three times the single fatal dose—in one hour and thirteen minutes, the last dose being equal to the minimum fatal dose. Recovery was as prompt after the last dose as after any of the preceding. This indicates that the drug was fixed or destroyed at the rate of one fatal dose every twenty minutes.

Protocol 6. Cat, female, 2.55 kg.

2:32 p. m.: Start injection of beta-eucain, 1—1000, into femoral vein.

3:05 p. m.: 38 c.c. total injected; no symptoms.

3:25 p. m.: 60 c.c. total injected; slight convulsive movements.

3:40 p. m.: 83.7 c.c. total injected; no increase in symptoms.

3:45 p. m.: Animal released; stands, but is somewhat unsteady from long restraint, otherwise seems normal.

In this case a total of 37.5 mg. $\times$ kg. of beta-eucain, or nearly four times the fatal vein dose, was injected in one hour and eight minutes without producing serious symptoms. The indicated rate of destruction or fixation is at least as rapid as one minimal fatal dose every twenty minutes.

The rates of destruction or elimination for the several drugs investigated seem to be about as follows: Alypin, beta-eucain, apothessin, nirvanin, procain, and stovain are destroyed at the rate of at least one fatal vein dose every twenty minutes. Holocain is apparently destroyed a little less rapidly, one fatal dose requiring about half an hour; while cocain is not destroyed more rapidly than about one fatal dose per hour, if as rapidly as that, and the rate of its destruction apparently decreases progressively.

In connection with the elimination of certain of the digitalis bodies we have ¹⁷ spoken of the elimination of a poison from those organs upon which its essential, or toxic, actions are exerted as the "essential" elimination to distinguish it from that elimination which may take place immediately after the drug enters the blood stream. In the case of these local anesthetics this "essential" elimination proceeds at a very rapid rate and is accomplished, as we shall show later, by destruction or fixation of the drug. Even in the case of cocain "essential" elimination is rapid at first, although relatively much less so than with the remaining local anesthetics. Unlike the digitalis bodies the "essential" elimination of the local anesthetics is virtually the same as the immediate elimination and is practically identical with total elimination, excepting in the cases of cocain and holocain.

This affords an excellent illustration of the difference between those drugs which show the so called "cumulation" and those which do not. In the case of the former the immediate elimination from the blood may proceed very rapidly but "essential" elimination is a slow process, so that the administration of repeated doses leads to an increasing amount of the drug which

17. Hatcher, Robert A., and Eggleston Cary: Jour. Pharm. and Exp. Therap. **12**: 405, 1919.

is present and acting upon the essential organs. In the case of the latter the administration of repeated doses finds the organs on which the essential actions are exerted fully restored to normal and quite free from remaining fractions of the previous dose or doses.

5. TOXICITY BY SUBCUTANEOUS INJECTION

The relative toxicity of several of the local anesthetics by subcutaneous injection has been studied by LeBrocq,¹⁸ Chadbourne,¹⁹ Grode,²⁰ by Schroeder, and by Impens. We have prepared Table 1 from the figures given by these authors for the toxicity in rabbits.

TABLE 1.—SHOWING THE FATAL SUBCUTANEOUS DOSES FOR RABBITS OF ALYPIN, COCAIN AND TROPACOCAIN, ACCORDING TO VARIOUS AUTHORS

Anesthetic	Fatal Dose, Mg. × Kg.	Author
Alypin.....	115-147	LeBrocq
	110	Schroeder
	50	Impens
Cocain.....	200	Chadbourne
	153-192	LeBrocq
	100-150	Impens
	87-100	Grode
Tropacocain.....	over 500	Chadbourne
	281-345	LeBrocq

Examination of the various doses stated as fatal for the rabbit after subcutaneous injection of cocain, tropacocain and alypin shows a wide range of variation, the largest dose stated for cocain being more than twice the smaller; the largest for tropacocain more than one and three-fourths times as great as the smallest; and the largest for alypin being nearly three times the smallest. Three possible explanations may be offered for such divergence:

1. Variation in the susceptibility of the animals.
2. Variation in the concentrations of the solutions used.
3. Variation in the rates of absorption from the subcutaneous tissues.

18. LeBrocq, C. N.. Brit. Med. Jour. **1**: 783, 1909.

19. Chadbourne, Arthur P.: Brit. Med. Jour. **2**: 402, 1892.

20. Grode, Julius: Arch. exper. Path. u. Pharm. **67**: 122, 1912.

We are inclined to believe that in view of the rapidity of the destruction of these drugs the latter is the probable explanation. In fact, our observations on the rate of destruction after intravenous injection led us to abandon the effort to fix accurately the fatal subcutaneous doses for the several local anesthetics. We therefore confined these observations to a few experiments with each drug to observe the symptoms produced, the approximate rates of absorption and destruction, and to determine whether or not the relation of destruction to absorption was such as to enable the cat to survive at least five times the average fatal vein dose when injected subcutaneously. A few protocols will illustrate the results obtained.

Protocol 7. Cat, female, 3.34 kg.

9:40 a. m.: 60 mg. $\times$ kg. cocain in 10 per cent. solution subcutaneously.

9:48 a. m.: Emesis.

5:57 a. m.: Clonic convulsions; heart slow and strong; respiration only moderately stimulated . . . convulsions repeated nearly continuously.

10:51 a. m.: Convulsions over.

10:57 a. m.: Heart very slow; respiration stops for periods and then returns; is very slow and feeble.

11:07 a. m.: Respiration stopped.

11:10 a. m.: Heart stopped.

Protocol 8. Cat, female, 3.16 kg.

9:45 a. m.: 100 mg. $\times$ kg. apothetin in 10 per cent. solution subcutaneously.

9:58 a. m.: Moderate motor excitation.

9:59 a. m.: Violent general clonic convulsion lasting more than one minute; not repeated.

10:02 a. m.: Animal able to walk but still shows some motor excitation like that seen from morphin.

12:00 m.: Seems quite normal.

Protocol 9. Cat, female 2.28 kg.

One hundred and twenty-five mg. $\times$ kg. tropacocain in 10 per cent. solution subcutaneously. No symptoms developed.

Each of the drugs was tried in this way and five, or more than five, times the minimal fatal vein dose was survived when injected subcutaneously in the case of alypin, apothetin, beta-eucain, nirvanin, procain, stovain, and tropacocain. Three and one-third times the fatal vein dose of cocain was survived after subcutaneous injection, while four times the fatal vein dose led promptly to death. Holocain showed a similar high toxicity by subcutaneous injection. It will be recalled that in an earlier section we stated that both holocain and cocain were less rapidly destroyed after intravenous injection than were the other members of the group. Both of these, however, are absorbed rapidly from the subcutaneous tissues so that the rate of absorption may exceed that of destruction with the result that the animal is fatally poisoned by a relatively small dose injected subcutaneously. Absorption of all the other members of the group, though rapid, does not keep pace with the capacity for destruction so that five or more times the fatal vein dose can be administered subcutaneously at one injection without causing death.

With the exceptions of one dose of 150 mg. $\times$ kg. of stovain and one of 50 mg. $\times$ kg. of cocain, the time between the injection of the drug and the appearance of the first symptom—where any effects at all were produced—averaged just over twelve minutes, with a minimum of seven and a maximum of sixteen minutes. After the dose of stovain cited as an exception the interval was twenty-five minutes, while after that of cocain it was twenty-eight minutes. A dose of 60 mg. $\times$ kg. of cocain, however, was followed in eight minutes by the first symptoms of its systemic action. When the doses given produced convulsions as part of their toxic actions the average interval between the administration of the dose and their onset was a little more than twenty-two minutes, with a minimum of nine and a maximum of eighty minutes. The eighty-minute interval occurred following the dose of stovain just cited as requiring twenty-five minutes before the appearance of the first symptom and is the

only one greater than twenty-five minutes. If it be omitted from the calculation of the average time to convulsions, the latter falls to fourteen minutes. One instance has not been included in either of the preceding computations, namely, that of a dose of 50 mg. $\times$ kg. of cocain. In this case no convulsions were observed in five hours, but more than twenty-seven hours later the animal had one mild clonic convulsion. This experiment is so strikingly at variance with all of the others that we append the protocol.

Protocol 10. Cat, female, 2.6 kg.

February 14, 10:26 a. m.: 50 mg. $\times$ kg. cocain in 5 per cent. solution subcutaneously. 10:54 a. m.: Emesis, repeated. 2:44 p. m.: Emesis. 5:00 p. m.: Animal has been depressed all afternoon.

February 15, 9:00 a. m.: Still somewhat depressed, refuses food. 2:00 p. m.: Mild clonic convulsion; not repeated.

February 16, 9:00 a. m.: Apparently normal.

In this experiment absorption began fairly promptly for emesis appeared twenty-eight minutes after the injection, and continued more slowly, for emesis was repeated at intervals for the next four hours. It is quite evident, however, that slow as was the absorption, "essential" elimination was not able to keep pace with it for a clonic convulsion appeared twenty-seven hours after the injection, and we have not seen convulsions develop after intravenous injection of less than about two-thirds of the fatal dose. In this instance "essential" elimination was much slower than one fatal vein dose per hour, as stated to be the usual approximate rate after intravenous injection, and illustrates the difference between cocain and the other members of the group.

Reference to the review of the literature of intoxication in man shows that the symptoms are much the same as those here recorded for cats; that they do not differ materially in nature for the several drugs; that the interval between the injection of the drug and the onset of symptoms is generally very brief in man, as in animals; and, finally, that recovery in man is quite

rapid with all the drugs except that after cocain it may be decidedly prolonged, just as in the case of cats. Thus after all of the drugs used, except cocain, recovery in man was almost invariably complete in an average of a little over two hours, the limits being half an hour and three hours and forty minutes. No attempt has been made to fix the limit of the several time intervals for cats with great accuracy, gross differences between the several members of the group being alone of importance. Therefore the figures stated are not to be taken as exact indexes of either the rapidity of absorption or of duration of action. Such a determination would have required far larger amounts of most of the drugs than we had at our disposal, and would have added little, if anything, of importance to the results.

We did, however, pursue this phase of the investigation further by observing the effects of giving repeated subcutaneous doses of several of the drugs at intervals of fifteen minutes. The results are shown in the accompanying protocols.

Protocol 11. Cat, male, 2.16 kg.

10:06 a. m.: 10 mg. $\times$ kg. cocain in 10 per cent. solution subcutaneously; dose repeated at 10:19 and 10:34 a. m.

10:39 a. m.: Emesis, depression, crying, tremulous.

10:48 a. m.: Mild general convulsions, not repeated.

11:30 a. m.: Seems fairly normal, sits up.

3:00 p. m.: Eats ravenously, is entirely normal.

Protocol 12. Cat, female, 2.60 kg.

10:06 a. m.: 10 mg. $\times$ kg. cocain in 10 per cent. solution subcutaneously; dose repeated at 10:21 and 10:36 a. m.

10:41 a. m.: Nausea, slightly tremulous, pupils dilated.

10:51 a. m.: 10 mg. $\times$ kg. as before.

11:02 a. m.: Very hyperexcitable, almost in convulsions.

11:21 a. m.: 10 mg. $\times$ kg. as before, no convulsions having developed.

11:30 a. m.: Mild general clonic spasm, followed by tetanic spasm.

11:56 a. m.: Spasms repeated up to now; animal now sitting up, head shakes continuously.

2:50 p. m.: Emesis; is depressed.

4:00 p. m.: Still depressed, refuses food, no other symptoms. Normal following morning.

Protocol 13. Cat, female, 3.60 kg.

10:00 a. m.: 20 mg. $\times$ kg. apothecin in 10 per cent. solution subcutaneously. Dose repeated at 10:15, 10:30, 10:45, 11:00, 11:30 and 11:45 a. m. and at 12:00 noon. Slightly tremulous and showed slight motor excitation after the fifth and seventh doses.

12:15 p. m.: 30 mg. $\times$ kg. as before.

12:21 p. m.: Slightly tremulous, otherwise normal. No further symptoms.

These protocols illustrate the differences already mentioned between the relative rates of absorption and destruction of holocain and cocain as compared with other of the local anesthetics, including nirvanin, procain and stovain, protocols of which have been omitted. The subcutaneous administration of only one and one-half times the fatal vein dose of holocain in three equal portions during thirty minutes was followed by a convulsion, and recovery seemed about complete an hour after the last dose. Three and one-third times the fatal vein dose of cocain, in five equal doses in one hour and fifteen minutes, produced serious intoxication and recovery was not complete after a lapse of four hours following the last dose.

A further, and striking, illustration of the slow "essential" elimination of cocain is to be found in Grode's paper. He tried to habituate various animals to cocain by repeated subcutaneous injection and he noted, in both cats and dogs, what he called an increased susceptibility to the later repetitions of a fixed dose, when these were made at intervals of one or two days. Thus, in one cat to which he attempted to give a daily subcutaneous dose of 20 mg. $\times$ kg. of cocain, the first dose caused salivation only; the second caused emesis; the third, three tetanic spasms; the fifth, eight tetanic spasms; then a two day interval

intervened between the sixth and seventh doses, but the seventh dose caused repeated spasms and the eighth, on the following day, proved fatal in fifteen minutes.

In this experiment of Grode's it is to be noted that the cat was unable to destroy or eliminate as much as one and one-third times the fatal vein dose in the course of twenty-four hours when injected subcutaneously. This is a much slower rate of "essential" elimination than was indicated by our experiments in which cocain was injected intravenously, and the difference is not readily explained.

In contrast with these we have administered about seven times the fatal vein dose of nirvanin in one and three-quarter hours, nine times the fatal vein dose of procain in two hours, and nine and one-half times the fatal vein dose of apothesis in two and a quarter hours, without the production of convulsions in any instance. In all of these experiments, even after such very large doses, recovery was essentially complete within three hours and a half.

6. INFLUENCE OF EPINEPHRIN ON TOXICITY BY SUBCUTANEOUS INJECTION

The results of the experiments discussed in the preceding section made us feel quite certain that the simultaneous injection of epinephrin and any of the local anesthetics would delay the onset of symptoms and diminish the toxicity of a given dose of the anesthetic by virtue of delayed absorption.

The results of all these experiments may be briefly summarized by saying that no symptoms followed doses of 100 and 150 mg. $\times$ kg. of narvanin, 100 mg. $\times$ kg. of apothesis, or 50 mg. $\times$ kg. of alypin when injected subcutaneously in solutions containing 0.05 mg. $\times$ kg. of epinephrin in 1:10,000 dilution. A dose of 150 mg. $\times$ kg. of stovain with epinephrin caused nausea and emesis only, and they did not appear until one hour after the injection. A dose of 50 mg. $\times$ kg. of holocain with epinephrin was followed in thirteen minutes by the onset of violent clonic convulsions which were repeated many times during about five

hours. The animal, however, recovered completely during the night and was apparently normal on the following morning. We have previously shown that the simultaneous administration of epinephrin greatly reduces the toxicity of procain by subcutaneous injection. In one experiment with alypin, the dose duplicating that previously mentioned, emesis followed thirty-seven minutes after the injection and clonic convulsions appeared two minutes later and were repeated for nearly an hour and a half. It is not clear why the epinephrin failed to have a greater effect in delaying the absorption in this case, unless it be that this animal was more susceptible than usual to the irritant actions of the alypin. Finally, a dose of 85 mg. $\times$ kg. of cocain, with epinephrin, produced no symptoms in more than three hours, but the following day—eighteen and a half hours later—the animal was found in clonic convulsions which continued for four hours, up to the death of the cat.

These experiments also show that cocain and holocain are relatively slowly destroyed, so that even when absorption is delayed by the vasoconstrictor action of epinephrin they are still capable of accumulating in the essential organs to cause serious intoxication or death when five to six times the fatal vein dose is injected subcutaneously. On the other hand, the importance of the rapid destruction of the other members of the group is emphasized by these experiments with epinephrin, and it would seem to point to the decided value of always using epinephrin, as a routine, in combination with the cocain substitutes for subcutaneous injection in man to delay absorption and diminish the chances of accidental poisoning.

7. NOTES ON ABSORPTION FROM MUCOUS MEMBRANES

Some experiments were performed to determine whether or not the local anesthetics are rapidly absorbed from the mucous membranes, especially those of the nose, urethera and bladder. Cocain and procain were employed chiefly, though a few experiments were made with apothetin. At first we

attempted to apply a measured amount of a solution of procain to the cat's nasal mucous membrane by the insertion of a series of cotton plugs saturated with the solution. The small size of the nasal spaces, however, showed this method to be unsatisfactory and we therefore resorted to plugging the nares as far back as possible with small pledgets of cotton saturated with the solution to be tested. Then, with cat restrained in a box and held with the head upward, small drops of the solution were instilled at frequent intervals into each nasal cavity until the desired amount had been introduced, or until symptoms developed. The one objection to this method is that it does not entirely prevent some of the solution from running back into the pharynx and being swallowed. The following is a typical protocol.

Protocol 14. Cat, female, 2.8 kg.

9:32-9:39 a. m.: 53 mg. $\times$ kg. cocain in 10 per cent. solution instilled into nose.

9:45 a. m.: Tremulous, pupils dilated.

9:51 a. m.: Clonic convulsion; repeated several times.

9:58 a. m.: No further convulsions.

10:20 a. m.: No evident symptoms.

11:05 a. m.: Emesis.

1:00 p. m.: Apparently normal.

Repeated clonic convulsions followed immediately after the instillation of 212 mg. $\times$ kg. of procain in 20 per cent. solution into the nose of another cat, but the animal had recovered completely in two and a half hours. A third cat showed no symptoms after the instillation of 133 mg. $\times$ kg. of procain, partly into the nares and partly into the conjunctival sacs. In a fourth experiment 112 mg. $\times$ kg. of apothesis in 10 per cent. solution were instilled into the nose, and the cat vomited once about half an hour later, but showed no other symptoms. While absorption was sufficient to permit of the development of some evidences of intoxication in three of the four experiments, it must be borne in mind that the doses

employed were massive; thus the dose of cocain corresponds to 53 grains, or 3.5 grams, for a man of average size; that of procain which produced convulsions, to 212 grains, or nearly 14 grams; and that of apothessin, to 112 grains, or about 7 grams, for man. It is not surprising that some symptoms should have resulted from such doses, but rather that the intoxications produced were so mild as recorded.

Several instances of acute poisoning in man have been reported following the instillation of each of a number of the local anesthetics into the male urethra, and so frequent have been such cases following the use of cocain that special warning is given by many writers as to the dangers of thus anesthetizing the urethra. This would seem to indicate rapid absorption from the urethral mucosa, but it is noteworthy that among the six cases of poisoning in man after intraurethral instillation of beta-eucain or alypin, which we have cited from the literature, the urethral mucuous membrane had been damaged in four. Such a condition would naturally be expected to hasten the absorption, especially in such a highly vascular stricture, when the solution is introduced under considerable pressure, as is the case in most of such instillations. We undertook to study the absorption of some of these drugs from the urethra of the male cat, but found its capacity too small, as shown by the protocol of a control experiment.

Protocol 15. Cat, male, 2.16 kg. (control)

Lightly anesthetized with ether, bladder exposed, blunt needle tied into anterior urethra and a concentrated solution of methylene blue instilled slowly. When 0.1 c.c. of the solution had been injected it was seen to appear in the bladder, showing the small capacity of the cat's urethra.

It was evident from this experiment that large doses of the local anesthetics could not be kept within the cat's urethra, even when very concentrated solutions were employed, and that the injection was chiefly into the bladder. Nevertheless, the solution was made to pass through the urethra, and some of it did remain

there, so we injected several cats in this way with large doses of the anesthetics. The results were invariably negative, that is, no absorption was evident in any of the seven experiments in which amounts of procain up to 150 mg. $\times$ kg.; of cocain, up to 90 mg. $\times$ kg.; and of apothessin, up to 100 mg. $\times$ kg. were thus instilled through the urethra into the previously emptied bladder. The entirely negative results of these experiments led us to abandon their further repetition.

We performed one experiment to discover whether the cat would absorb appreciable amounts of procain from the vagina. A blunt needle was inserted into the vagina of an unanesthetized cat and the orifice was plugged with cotton saturated with petrolatum. Five hundred milligrams, or 277 mg. $\times$ kg., of procain in 50 per cent. solution were instilled. No symptoms resulted.

These experiments on absorption from the mucous membranes indicate: That the local anesthetics are not more rapidly absorbed from the nasopharyngeal mucous membranes of the cat than from the subcutaneous tissues. That in the cat, the bladder, urethra and vagina absorb the local anesthetics very poorly.

8. ELIMINATION BY THE LIVER

Weichowski²¹ sought to determine the fate of cocain in the animal organism by administering large doses orally to dogs and rabbits and examining the urine for cocain and ecgonin after collection for forty-eight hours. He found that dogs excreted an average of 5.1 per cent. of the dose administered, the amounts recovered in the urine varying between none and 12.3 per cent. Rabbits, on the other hand, excreted none at all in the urine. He added cocain to the hashed livers or muscles, freshly excised, and agitated the mixtures for periods of four hours at the end of which he recovered about 80 per cent. of the cocain originally added. From these experiments he concluded that the

21. Wiechowski, Wilhelm: Arch. exper. Path. u. Pharm. **46**: 155, 1901.

destruction of cocain should be regarded as a vital phenomenon. He also extracted the liver of a dog, weighing 5.8 kg., which died half an hour after the administration of a total of 0.3744 gm. of pure cocain. One hundred and eighty-eight grams of the liver yielded 8 mg. of cocain, an amount which corresponded to that which would have been present after uniform distribution of the drug throughout the body. He further determined that ecgonin does not appear in the urine of the dog or rabbit in appreciable amounts as a decomposition product of cocain.

Rifâtwachdani²² obtained quite different results for the excretion of cocain in the urine of rabbits after subcutaneous injection. He found that rabbits excreted in the urine in twenty-four hours amounts varying from 42 to 85 per cent. of a single subcutaneous dose of 50 mg. of cocain hydrochlorid, showing absence of marked destruction. The excretion of cocain was studied in one rabbit receiving a daily subcutaneous dose of 50 mg. of cocain. The amounts recovered in the urine on successive days rose from 33.6 to 112.1 per cent. of the daily dose. In the whole experiment 65.8 per cent. of the cocain administered was thus excreted. No ecgonin was found in the urine. The author also cites and confirms Kohlhardt's observation that long contact of cocain with the tissues, as by injection into a leg the circulation of which had been interfered with by constriction with a tourniquet, leads to a great reduction in its toxicity when the constriction is released. He tried to extract the cocain from such previously constricted legs, amputated two or three hours after the injection, and recovered from 20 to 100 per cent of that injected. He holds that this shows conclusively that there is no destruction or detoxification of cocain by the tissues, and suggests that the reduction in toxicity following injection into the constricted leg may be due to slow absorption and entrance into the general circulation, as is known to be the case with strychnin.

22. Rifâtwachdani, Suleiman: *Biochem. Ztschr.* **54**: 83, 1913.

We showed in a previous paper that procain is rapidly destroyed during its perfusion through the cat's liver, and the same was shown to be true of stovain by Smith and Hatcher. In these two papers it was also shown that neither procain nor stovain is excreted in the urine of the cat in appreciable amounts. The results of the perfusion experiments were so clear cut and definite in demonstrating the destruction or fixation of these two local anesthetics by the liver that we decided to study the fate of several other members of the group in the same way. Two abbreviated protocols illustrate the methods of procedure and the results obtained.

Protocol 16. Cat, female, 3.44 kg.

Under light ether anesthesia the animal was bled from the carotid artery while normal saline solution was being injected into a femoral vein. After defibrination the diluted blood measured 200 c.c. In this we dissolved 600 mg. of cocain hydrochlorid and divided the whole into a portion measuring 20 c.c., which was reserved for control tests, and one measuring 180 c.c., which was used for perfusing the liver.

The liver of the cat was removed at once after exsanguination, a cannula was tied into the portal vein and all the remaining blood was washed out with fresh, warm normal saline.

The liver was then perfused with the 180 c.c. of diluted defibrinated blood, containing 540 mg. of cocain, for one hour at a temperature of 37 C. The perfusion fluid passed through the liver more than thirty-five times, being oxygenated frequently in the intervals.

After perfusion 180 c.c. of fluid were recovered, some dilution of the original having taken place with the saline which remained in the liver after washing.

Both the perfused fluid and the sample of defibrinated, diluted blood which was reserved for control were tested on cats by intravenous injection.

Tests

Control blood: Cat, male, 2.58 kg.

11:20 a. m.: 6 c.c. $\times$ kg. of the control blood were injected rapidly into femoral vein. Immediate respiratory stimulation, violent clonic convulsions, repeated every few minutes until 11:40 a. m.

11:50 a. m.: Killed with chloroform.

Perfused fluid: Cat, male, 2.12 kg.

11:27-11:28 a. m.: 7.78 c.c. $\times$ kg. perfused fluid injected into femoral vein. Respiratory stimulation, one clonic convulsion.

11:29-11:31 a. m.: 6.83 c.c. $\times$ kg. additional injected. One clonic convulsion.

11:33-11:34 a. m.: 4.69 $\times$ kg. additional injected. Violent clonic convulsions began and were repeated just as in the control test.

Calculation

6.0 c.c. of control blood contained 15 mg. cocain.

19.3 c.c. of perfused fluid, equivalent to 6.0 c.c. of control, contains 15 mg. of cocain.

180.0 c.c. of perfused fluid contains 138.6 mg. cocain.

Since 540 mg. of cocain were originally present in the 180 c.c., 401 mg. were destroyed in the liver during perfusion.

Since the original perfusion fluid was somewhat diluted during passage through the liver, this figure is a little too high, but the result of the experiment is to show conclusively that the liver destroyed or fixed not less than half of a total of 540 mg. of cocain during perfusion for one hour, or an amount corresponding to about five fatal intravenous doses as for the cat from which the liver was removed.

Protocol 17. Cat, male, 3.65 kg.

Bled and liver prepared as in preceding, and 1 gm. of tropacocain, dissolved in a little normal saline, was added to the defibrinated blood and the whole made up to 250 c.c. Portion of 50 c.c. reserved for control tests; 200 c.c. used to perfuse the liver.

The perfusion was carried on for half an hour, the fluid flowing through the liver as fast as possible. At least forty complete circuits were made.

A test of the control fluid showed no destruction, while one of the perfused fluid indicated practically total destruction of the entire 800 mg. of tropacocain originally present, or an amount equivalent to more than ten times the single fatal vein dose for the animal from which the liver was removed.

The unused portion of the control sample of defibrinated blood was extracted and the extract tested on a cat. The test indicated recovery of practically all of the tropacocain originally present.

The liver, after perfusion, was then ground and extracted by the same method as that employed for the blood and tested on a cat. The test of the extract corresponding to one-third of the entire liver failed to reveal the presence of any tropacocain.

Perfusions similar to these were carried out with apothecin and nirvanin and showed that practically all of a total of 500 mg. of apothecin and about two-thirds of a total of 900 mg. of nirvanin were destroyed²³ by the liver during perfusion for one hour. In all, six of the nine different local anesthetics have been tested by perfusion through the excised liver and in every case it has been demonstrated that that organ is capable of destroying large amounts of these drugs rapidly. It is scarcely necessary to remark that we made control experiments to exclude destruction or other form of retoxification of the drugs by defibrinated, diluted blood, kept at body temperature and oxygenated during periods of time similar to those elapsing in the perfusions.

This rapid destruction of the local anesthetics by the liver accounts not only for the rapid recovery of the intact animal from the intravenous injection of doses which are just sublethal, but also for the fact that with most of the members of the group such doses can be repeated indefinitely at intervals of about twenty minutes until many times the single fatal dose has been injected. This destruction also explains the fact that when just sublethal doses are injected intravenously, even though respiration may have been stopped for one or two minutes, the animal will recover if the heart continues to beat and circulate the blood through the liver. The rapid destruction of these drugs also serves to explain in part the success of some of the resuscitative measures to be

23. We do not know with absolute certainty that the local anesthetics are actually destroyed in the liver, in the sense of chemical decomposition. There is a possibility that they may merely be fixed in an insoluble, or inert, form. All of the evidence, however, points to actual destruction by decomposition and we therefore prefer to speak of these drugs as being destroyed.

described later, as well as the ultimate recovery from more than average lethal doses after the previous administration of a cardiac stimulant drug.

It seems certain that man is also capable of rapidly destroying the anesthetics in his liver and that this largely accounts for his rapid recovery from severely toxic symptoms. Such destruction is a matter of prime importance in connection with the employment of antagonistic and resuscitative measures in cases of acute intoxication in man, for it is evident that if the vital functions of respiration and circulation can be maintained for even a short time there is always the chance of complete recovery through destruction of a portion of the drug which has been absorbed.

9. EFFORTS TO INFLUENCE THE TOXICITY OF THE LOCAL ANESTHETICS

One of the principal objects of our investigation of the local anesthetics having been the endeavor to throw some light on the factors which favor acute intoxication in man and the methods of avoiding or overcoming accidental poisoning, we systematically investigated a number of agents and measures which might be anticipated to exert some influence on the toxicity of these drugs. In many instances one or two experiments were sufficient to convince us that a lead was not worth following, so that we abandoned further work at once. In others we felt that we should make our experiments somewhat more extensive and searching, even though the results at first were negative

(a) *Influence of Atropin.*—We performed a series of thirteen experiments to determine whether or not the intravenous injection of atropin just before that of one of the anesthetics would influence the toxicity of the latter through paralysis of the vagus endings in the heart or stimulation of the respiratory center. The dose of atropin employed was either 2 to 5 mg. $\times$ kg. and the interval between its injection and that of the local anesthetic varied from three to ten minutes. Cocain, stovain, procain and apothessin were

tried as representatives of the group, doses both larger and smaller than those stated as the average fatal were administered. There was no evidence of any influence of atropin upon the symptoms produced by the local anesthetics, or upon the size of the fatal vein dose.

(b) *Influence of Sodium Bicarbonate.*—Gros²⁴ showed that the addition of sodium bicarbonate to the commonly used salts of the anesthetic bases enhanced their anesthetic activity, due, he believed, to the better penetration of the nerves by the liberated bases than by their salts. Sollmann²⁵ summarizes a series of his own investigations and generally confirms Gros so far as the increase of anesthetic activity by the addition of sodium bicarbonate is concerned, but states that it is not certain whether such addition modifies their toxicity or not. We therefore investigated the influence of sodium bicarbonate on the toxicity of the various drugs of the group.

In all of the experiments the mixture of the local anesthetic with the sodium bicarbonate was made in the syringe just before injection, or in a graduate, and the solution, which usually showed some cloudiness or a fine precipitate, was vigorously shaken and drawn into the syringe for immediate injection. In every instance the dose of the bicarbonate was 25 mg. $\times$ kg. in the form of a 5 per cent. solution, while the local anesthetic was used in the customary concentration. All injections were made intravenously at the usual rate. The doses of the local anesthetics were usually 75 per cent. of the average fatal, though in some cases the fatal doses were used, and once or twice doses larger than the average fatal were employed to determine whether there was a reduction in toxicity. In a few experiments repeated large doses of the anesthetic were given along with the alkali. The results, including the number of experiments performed, are shown in Table 2.

24. Gros, O.: Arch. exper. Path. u. Pharm. **62**: 380, 1910, and **62**: 80.

25. Sollmann, Torald: J. A. M. A. **70**: 216, 1918.

(c) *Influence of Caffein.*—Caffein has been employed clinically in the treatment of acute cocain poisoning and Bastedo²³ cites Lieb as having found caffein effective in removing cocain heart block in the isolated turtle heart. It seemed possible, therefore, that it might prove somewhat antagonistic to the toxic actions of the local anesthetics in cats. Doses of 10 mg. $\times$ kg. of caffein were slowly injected intravenously and three minutes later one and a quarter times the minimal fatal dose of the local anesthetic to be tested was injected in the usual manner. Cocain, procain and apothecin were tried, but in no instance was there any evidence of antagonism, even when artificial respira-

TABLE 2.—SHOWING RESULTS OF SIMULTANEOUS INTRAVENOUS INJECTION OF LOCAL ANESTHETICS WITH 25 MG. KG. OF SODIUM BICARBONATE IN CATS

Anesthetic	Number of Experiments	Doses of Anesthetic per Cent. of Fatal	Effect of Toxicity
Procain.....	6	60-120	None
Nirvanin.....	3	80-100	None
Cocain.....	3	80-100	None
Apothesin.....	2	75	No increase
Stovain, beta-eucain, alpin, tropacocain	1 each	75	No increase

tion was employed to give the heart a chance to recover, nor was there alteration in the typical response to the local anesthetic. In one experiment we injected 3 mg. $\times$ kg. of caffein and 50 mg. $\times$ kg. of procain in the same solution with the usual prompt onset of symptoms, followed by death in the typical manner.

(d) *Influence of Hydrated Chloral.*—We noted in our previous paper two instances in which cats died promptly after the intravenous injection of as little as 9 and 10 mg. $\times$ kg., respectively, of procain, given during chloral narcosis. This suggested that hydrated chloral increases the toxicity of the local anesthetics,

26. Bastedo, Walter A.: *Materia Medica, Pharmacology and Therapeutics*, 2nd ed., 1919, p. 419, Saunders, Philadelphia.

hence we decided to investigate the matter further. A series of nineteen experiments were performed and the results are shown in Table 3.

TABLE 3.—SHOWING INFLUENCE OF HYDRATED CHLORAL ON TOXICITY OF THE LOCAL ANESTHETICS FOR THE CAT

Anesthetic	Dose		Rate of Injection	Narcosis*	Result
	Mg. × Kg.	% of Fatal			
Procain.....	9.0	21	5 min.	Deep	Death
	10.0	24	Rapid	Deep	Death
	30.0	71	Rapid	Moderate	Recovery
	48.9	119	28 min.	Deep	Recovery
	106.0	252	128 min.	Deep	Recovery
Cocain.....	10.0	66	Rapid	Deep	Recovery
	10.0	66	Rapid	Deep	Recovery
	12.5	83	Rapid	Deep	Death
Tropacocain.....	15.0	75	Rapid	Mild	Recovery
	15.0	75	Rapid	Mild	Death
	15.0	75	Rapid	Mild	Death
Stovain.....	25.0	99	Rapid	Moderate	Death
	27.0	100	Rapid	Moderate	Death

The following cats received repeated doses, all injections rapid:

Cat	Anesthetic	Dose		Interval in Min.	Narcosis	Result
		Mg. × Kg.	% of Fatal			
A	Apothesin.....	10.0	50	..	Mild	Recovery
		10.0	50	20		Recovery
		5.0	25	20		Recovery
B	Apothesin.....	10.0	50	..	Mild	Recovery†
		10.0	50	15		Recovery
		15.0	75	15		Recovery
C	Apothesin.....	15.0	75	..	Mild	Recovery
		15.0	75	20		Recovery
		15.0	75	20		Recovery
D	Stovain.....	20.0	72	..	Deep	Recovery
	Procain.....	35.0	83	20	Deep	Death
E	Stovain.....	20.0	72	..	Moderate	Recovery
	Apothesin.....	15.0	75	33		Recovery
F	Apothesin.....	15.0	75	..	Moderate	Recovery
	Procain.....	35.0	83	30		Recovery

* Deep narcosis is of such a degree that the animal cannot be roused; moderate is such that the animal can be roused partially by very rough handling and repeated painful stimuli, but lapses into sleep at once when left; mild is such that the animal remains in deep sleep when undisturbed, but can be roused fairly readily by rough handling, returning to deep sleep only after an interval of a few minutes.

In this connection we wish to emphasize the degree of intoxication produced by the chloral, even when classed as mild. Its severity is indicated by the fact that of 30 cats to which chloral was administered to produce narcosis, five died in their cages before the time for use and four died as a result of the handling incident to the operation.

† This animal received chloral the day preceding the injection of the anesthetic and was used without a further dose of chloral because it remained in a state of moderate to mild narcosis.

These experiments indicate that severe poisoning by hydrated chloral may increase the susceptibility of the cat to the toxic actions of the local anesthetics, occasionally to a marked degree. On the other hand, degrees of poisoning by chloral short of the most severe do not as a rule seem to have much influence on the susceptibility to the local anesthetics. That the previous administration of hydrated chloral does exert some influence both on the immediate response to the local anesthetics, and on the rate of recovery is, however, clearly shown by the results of two parallel experiments, illustrated by Figures 4 and 5. The figures carry all the data except the time required for complete recovery in the chloralized cat, which were approximately ten minutes after each of the three doses, contrasting with periods of less than three minutes in the normal cat. The tracings show that after chloral the fall in blood pressure following the injection of the anesthetic is decidedly less abrupt than in the normal control, and while this effect was not always seen, it still was of frequent occurrence in those experiments in which we took graphic records. The slower recovery is almost certainly due to interference with the circulation whereby a longer time is required for destruction in the liver.

It is surprising that chloral did not show a more decided influence on the ultimate recovery from large doses of the local anesthetics, or upon the size of the fatal doses, in view of the material delay in the initial rate of recovery as shown in the blood pressure curves, and in view of the lower initial blood pressure in the chloralized than in the normal cat.

(c) *Influence of Hemorrhage*.—Attention has been called by several clinical writers to the fact that acute intoxication by the local anesthetics seems to be rather more likely to occur in ill-nourished, anemic persons than in the robust. A perusal of the literature, of accidental acute poisoning, however, does not show that the anemic and ill-nourished supply any more such occurrences than do others. Nevertheless, we investigated the influence of severe acute hemorrhage on the

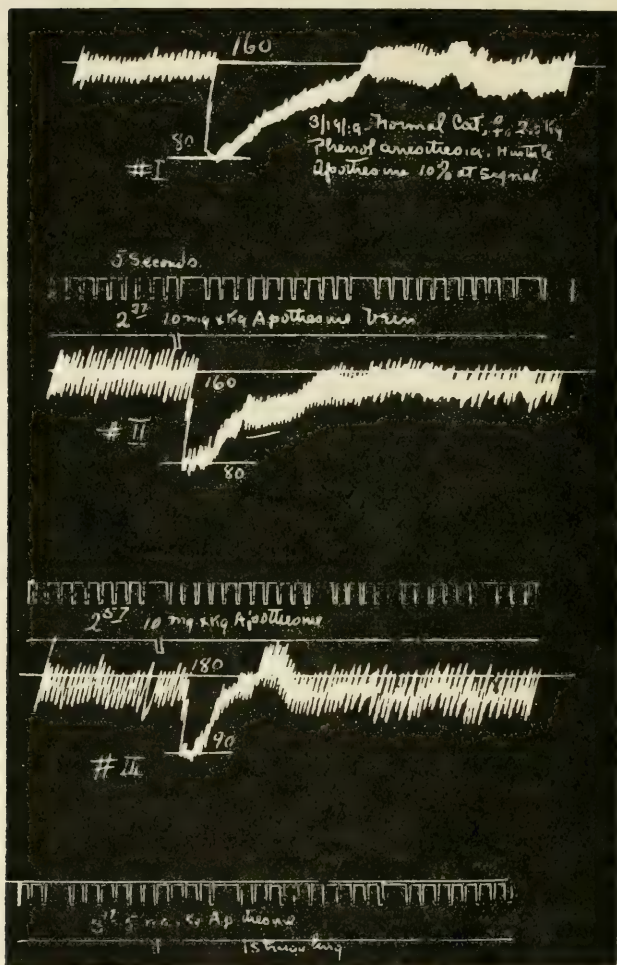


Fig. 4.

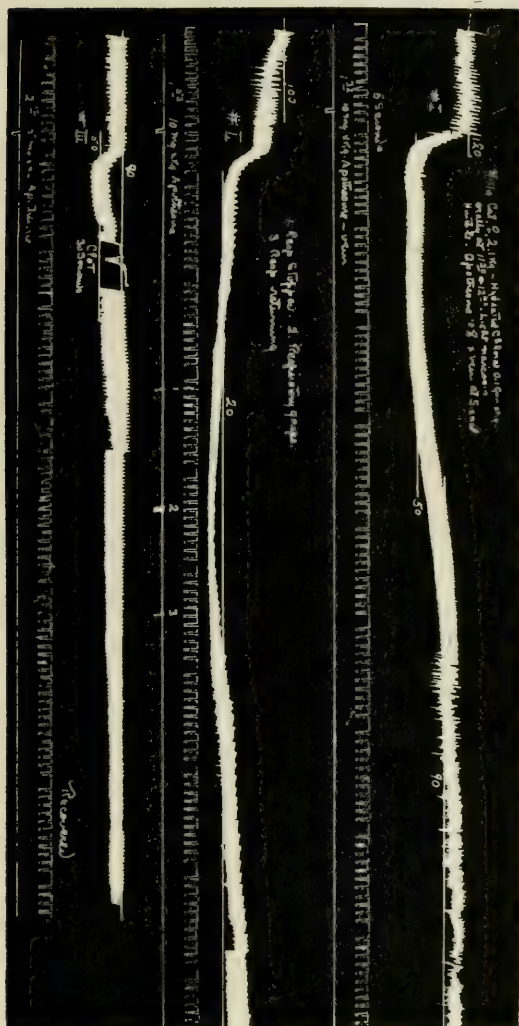


Fig. 5.

toxicity of several of these drugs by intravenous injection. The experimental method consisted in bleeding the cat from an artery to the extent of one fourth of the total calculated blood volume. In from two to five minutes after the bleeding the anesthetic was injected into the femoral vein. The general results are shown in Table 4.

TABLE 4.—SHOWING THE RESULTS OF INTRAVENOUS INJECTION OF LOCAL ANESTHETICS IN CATS PREVIOUSLY DEPRIVED OF ONE-FOURTH OF THEIR TOTAL BLOOD VOLUME

Anesthetic	Dose		Result
	Mg. × Kg.	% of Fatal	
Cocain.....	12.5	83	Typical symptoms; death
	12.0	80	Typical symptoms; death
	12.0	80	Typical symptoms; recovery
	11.0	73	Typical symptoms; death
Apothesin.....	20.0	100	Typical symptoms; death
	15.0	75	Typical symptoms; recovery
Stovain.....	22.5	89	Typical symptoms; death
	20.0	72	Typical symptoms; recovery
Tropacocain.....	15.0	75	Typical symptoms; death
	15.0	75	Typical symptoms; death
	10.0	50	Typical symptoms; recovery

Each of the following cats received two doses:

Cat	Anesthetic	Dose		Interval in Min.	
		Mg. × Kg.	% of Fatal		
A	Apothesin.....	10.0	50	..	Typical symptoms; recovery
		15.0	75	20	Typical symptoms; recovery
B	Apothesin.....	15.0	75	..	Typical symptoms; recovery
		15.0	75	30	Typical symptoms; recovery
C	Stovain.....	18.0	64	..	Typical symptoms; recovery
		22.0	88	35	Typical symptoms; recovery

Of the eleven cats receiving from 75 to 80 per cent. of the fatal vein dose of the local anesthetics, seven died after typical symptoms and four recovered normally. Three cats receiving repeated injections, on the other hand, recovered from both doses in a manner indistinguishable from normal cats. It would seem, therefore, that severe acute hemorrhage may be regarded as diminishing the cat's ability to recover from large fractions of the average fatal vein dose of

the local anesthetics in about half the instances. It is surprising that the effect of such a measure as the rapid withdrawal of one-fourth of the cat's total blood is not greater, for such a procedure not only materially reduces the blood pressure, but owing to the reduced blood volume tends to impair the function of all of the organs including the heart, the central nervous system and the liver. Such impairment might naturally be expected to both increase the cat's susceptibility to the local anesthetics and to reduce its capacity for destroying them in the liver.

(f) *Miscellaneous*.—We planned to investigate the effects of nervous exhaustion in cats upon the toxicity of the anesthetics and sought to produce exhaustion by prolonged, intense excitement. We administered doses of 10, 10 and 20 mg. $\times$ kg. of procain at intervals of ten and fifteen minutes, respectively, to one such exhausted cat, but saw no definite evidence of an increased susceptibility to the drug. We then resorted to the administration of camphor in doses sufficient to produce violent clonic convulsions which were repeated frequently during periods of two or more hours. When the convulsions were becoming infrequent and greatly diminished in severity owing to exhaustion we administered various doses of the local anesthetics, including procain, cocain, stovain and tropacocain, but observed neither increased susceptibility from the exhaustion, nor increased tolerance from the stimulant actions of the camphor.

We also sought to test the influence of surgical shock but after a single experiment we abandoned further efforts. In this experiment the cat was anesthetized lightly with ether and all of the measures commonly employed for producing shock were applied during a period of three quarters of an hour, at the end of which time the cat's blood pressure had fallen only 28 per cent. The intravenous injection of 75 per cent. of the fatal dose of apothesis resulted in death after four minutes, the respiration continuing feebly for nearly two minutes after the heart had stopped. No convulsions were observed following the dose of

apothesis. While the result in this experiment is suggestive of an increased susceptibility due to the surgical shock, we did not feel justified in attempting its repetition owing to the difficulty in producing shock and to the extremely abnormal condition of the animals as a result of the manipulations.

Finally we sought to determine the influence of hypertonic saline in delaying the penetration of the local anesthetics into the cells and performed two experiments in which 25 c.c. $\times$ kg. of a 10 per cent. solution of absolute sodium sulphate were injected intravenously just before the injection of procain, but there was no indication that this measure had any influence on the toxicity of the anesthetic.

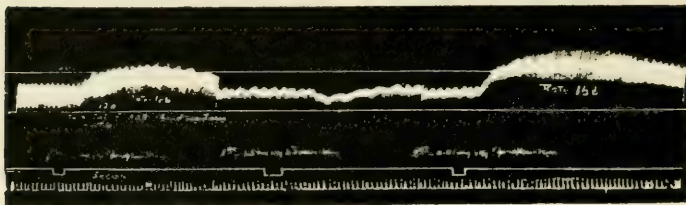


Fig. 6.

10. SYNERGISM WITH EPINEPHRIN ON SYSTEMIC ADMINISTRATION

It is well known that cocain and epinephrin have a synergistic action on the blood pressure, and we have called attention to the fact that procain is similarly synergistic with epinephrin, although, unlike cocain, it causes a fall in the blood pressure when given alone. All of the other substitutes for cocain which we have investigated also cause a fall in blood pressure, and produce local vasodilatation when injected subcutaneously. We have tried all of the remaining local anesthetics with reference to their synergism with epinephrin and have found that it is present, though not always as marked as with cocain and procain. The synergism is often absent when the doses of the cocain substitutes are sufficient to cause even a slight

initial fall of blood pressure, but when they are so small as to produce no appreciable effect, when given alone, the synergism with epinephrin is quite definite. It is not very marked with alypin and apothetin. The mechanism of this particular synergistic action is not understood and we hope to make it the subject of a more detailed investigation.

Figure 6 illustrates this synergism, and it will be noted that the rise produced by a fixed dose of epinephrin is not only greater, but is also more sustained after the administration of the anesthetic than before, a condition precisely analogous to that shown in the case of procain.

11. MEASURES OF RESUSCITATION

(a) *Artificial Respiration, With or Without Cardiac Massage.*—In the search for methods of resuscitation we thought first of employing artificial respiration, or cardiac massage, or both together. It was apparent that if these measures should prove effective they should be capable of preventing death in the cat after the injection of appreciably more than the average fatal dose or they would be of relatively little value in cases of intoxication in man. We had two reasons for adopting this view; first, because in cases of accidental poisoning in man the amount of drug absorbed cannot be determined and it may often be well above the minimum which would be fatal in a given individual. Second, because the rapid destruction of the anesthetics in the liver should promptly reduce the amount of the drug free to act on the heart and respiratory center to a point below that which would prove fatal, provided these two could be kept functioning for a few minutes.

Our method of procedure for determining the value of artificial respiration alone, or with cardiac massage, was to insert a tracheal cannula through a small incision after anesthetization of the skin with phenol, and to connect this with a respiration valve which was connected with an artificially interrupted air supply. We were thus ready to begin artificial respiration the

moment that spontaneous breathing stopped, and could carry it on efficiently and uniformly for as long as might be desired. The drug to be used was injected rapidly into the femoral vein. When cardiac massage was employed it was given manually by rapid rhythmic compression and relaxation of the soft thorax over the heart, and proved to be adequate to maintain a fair blood pressure even after the heart had stopped (see

TABLE 5.—SHOWING RESULTS OF APPLYING ARTIFICIAL RESPIRATION AND CARDIAC MASSAGE ON RECOVERY FROM LARGE DOSES OF LOCAL ANESTHETICS

Anesthetic	Dose		Resuscitative Measure	Result
	Mg.	% of Fatal		
Alypin.....	12.5	125	Artificial respiration.....	Death
Apothesin....	25.0	125	Artificial respiration.....	Recovery
	40.0	200	Artificial respiration.....	Death*
Beta-eucain..	15.6	125	Artificial respiration.....	Death
	18.7	150	Artificial respiration; cardiac massage	Death
Cocain.....	20.0	133	Artificial respiration; cardiac massage	Death
Holocain....	12.5	125	Artificial respiration.....	Death
	12.5	125	Artificial respiration; cardiac massage	Death
Nirvanin....	37.5	117	Artificial respiration.....	Death
	37.5	117	Artificial respiration; cardiac massage	Death
Procain.....	60.0	142	Artificial respiration.....	Death
	60.0	142	Artificial respiration.....	Death
	60.0	142	Artificial respiration.....	Death
	60.0	142	Artificial respiration.....	Death
	60.0	142	Artificial respiration.....	Death
Stovain.....	37.5	135	Artificial respiration.....	Death
	37.5	135	Artificial respiration; cardiac massage	Death
Tropaeocain.	25.0	125	Artificial respiration.....	Recovery

* This was the same animal which received the dose of 25 mg. $\times$ kg. of apothesisin one hour earlier.

tracings). We performed a series of eighteen experiments, the important points of which are shown in Table 5. In every instance the attempts to resuscitate were continued until we were certain that the animal would not recover, generally for at least ten minutes.

The results of this series of experiments show that neither artificial respiration alone, nor combined with cardiac massage, is an efficient method for the resuscitation of cats after doses of the local anesthetics varying between 117 and 200 per cent. of the fatal. In only

two of the eighteen experiments did the cat recover, and in neither was cardiac massage employed, the heart resuming spontaneously after brief temporary cessation. It would seem probable that these two cats were naturally more tolerant to the local anesthetics than general, such tolerance having been noted occasionally.

(b) *Influence of Epinephrin Plus Artificial respiration and Cardiac Massage.*—We stated in our earlier paper on procain that the simultaneous intravenous injection of epinephrin interfered to some extent with the toxic action of procain, and we there recorded one instance of a cat's surviving a dose of procain 50 per cent. larger than that usually fatal when it was injected simultaneously with epinephrin. Those observations suggested the further study of the value of epinephrin as an aid to resuscitation, and we performed a series of seventeen experiments which are illustrated by the following representative protocol and accompanying tracing—Figure 7.

Protocol 18. Cat, male, 3.34 kg.

Skin anesthetized with phenol and connection made with tracheal cannula for artificial respiration. Cannula into left femoral vein for injection of epinephrin in 1-100,000 solution. Normal tracing.

10:30 a. m.: 18.7 mg. $\times$ kg. beta-eucain into right femoral vein. Prompt cessation of respiration and heart, blood pressure fell to zero. Artificial respiration and injection of epinephrin begun at once. Cardiac massage twice to carry epinephrin to heart. Prompt recovery of heart; artificial respiration stopped after about three minutes. Complete recovery.

11:30 a. m.: 12.5 mg. $\times$ kg. beta-eucain as before. No attempt to resuscitate. Typical death, except for temporary partial recovery of heart.

The importance of carrying on artificial respiration in addition to the injection of epinephrin is illustrated by another experiment, the details of which are shown on the graphic record—Figure 8. This experiment shows the beginning recovery of the heart about two and one-half minutes after a dose of apothesis twice

that usually fatal. The heart then improved rapidly and the blood pressure rose above the level previous to injection. Cessation of artificial respiration was followed by a rapid fall in the blood pressure with weakening of the heart. Reinstitution of artificial respiration rapidly restored the heart. This phenom-

TABLE 6.—SHOWING EFFECTS ON TOXICITY OF LARGE DOSES OF THE LOCAL ANESTHETICS FROM THE INTRAVENOUS INJECTION OF EPINEPHRIN COMBINED WITH ARTIFICIAL RESPIRATION AND CARDIAC MASSAGE

Anesthetic	Dose		Epinephrin 1:100,000 C.c. × Kg.	Artificial Respira- tion	Cardiac Massage	Result
	Mg. × Kg.	% of Fatal				
Alypin.....	12.5	125	7.7	+	+	Recovery
Apothesin.....{	25.0	125	7.5	+	0	Recovery
	30.0	150	9.3	+	+	Recovery
	40.0	200	13.0	+	+	Recovery
Beta-eucain....	18.7	150	6.0	+	+	Recovery
Cocain.....{	20.0	133	8.0	+	0	Recovery
	22.5	150	8.1	+	+	Death
Holocain.....	12.5	125	9.4	+	0	Death*
Nirvanin.....	37.5	117	5.5	+	+	Recovery
Procain.....{	50.0	120	8.2	+	+	Death
	50.0	120	11.7	+	+	Recovery†
	60.0	142	8.0	+	+	Recovery
	80.0	190	9.0	+	+	Death‡
Stovain.....{	37.5	135	6.1	+	+	Death
	37.5	135	2.5	+	+	Recovery
Tropacocain..{	25.0	125	9.9	+	+	Recovery
	30.0	150	2.2	+	+	Death

* Recovery was apparently complete and resuscitative measures were stopped. Heart and respiration failed together five minutes later.

† This cat had received the dose of apothesin of 30 mg. × kg., recorded above, half an hour previously.

‡ Recovery was apparently complete in ten minutes and all resuscitative measures were stopped fifteen minutes after injection of the anesthetic. Spontaneous respiration was not resumed and heart began to fail after about one minute, but was restored promptly by reinstitution of artificial respiration. This was continued for nine minutes when spontaneous breathing began. Nine minutes later the animal died, the respiration failing before the heart. The cat in this experiment had received the dose of 60 mg. × kg. of procain, recorded above, only twenty-two minutes earlier.

enon was repeated once more, after which recovery was complete. Neither of the two temporary interruptions of artificial respiration was followed by the resumption of spontaneous breathing. This was probably the result of the direct action on the respiratory

center of the very large dose of apothessin, and was the probable cause of the beginning failure of the heart on both occasions.

The chief points in the several experiments in this series are shown in Table 6, from which it is evident that the combination of the intravenous injection of epinephrin with effective artificial respiration, with or without resort to cardiac massage, is usually capable of saving the cat from doses of the local anesthetics varying from 117 to 200 per cent. of the average fatal. Death resulted in only six of the seventeen experiments, and in one of these it followed the administration of nearly twice the fatal dose of the anesthetic—procain—to a cat which had received almost one and a half times the usual fatal dose of the same drug only twenty-two minutes previously. Control experiments showed that normal saline was without value in antagonizing the local anesthetics.

While cardiac massage cannot be carried out so efficiently in man as in the cat, it can be performed sufficiently to cause the greater part of an intravenous injection of epinephrin to reach the heart; and several satisfactory methods are available for carrying on artificial respiration in man. These measures, also, are all such as can be applied without loss of time under most conditions and they certainly seem to be the most effective means at our disposal for saving life after the development of symptoms of acute intoxication by the anesthetics of this group. Since acute poisoning in man is accidental and occurs when least expected, those who frequently employ these local anesthetics should always be prepared to apply the three resuscitative measures of intravenous infusion of epinephrin, artificial respiration and cardiac massage, and all three should be used in combination since no one alone is effective except in rare instances.

12. ANTAGONISM BY OUABAIN

The fact that the several local anesthetics promptly bring the cat's heart to standstill has been shown to be characteristic of their acute toxic action, but this paralysis, while immediate, is not permanent in all

cases, even after fatal doses, for we not infrequently observe a temporary, though rather feeble, spontaneous return of the heart beat, as illustrated in Figure 3. These facts, combined with the rapid destruction of the anesthetics by the liver, prompted us to try the effect on their toxicity of the previous stimulation of the heart by one of the digitalis bodies. For the sake of convenience we selected ouabain, and also because it has a fairly well fixed fatal dose on which we could base our calculations. We administered 60 per cent. of the fatal dose of ouabain to several cats, either intravenously or intramuscularly, and after intervals of fifteen minutes to several hours, gave more than an average fatal dose of one of the local anesthetics in the usual manner. Artificial respiration was employed in most cases to tide over the period required for recovery of the heart, for we found that without it the cat would die from the respiratory paralysis even when the heart was evidently recovering. Our first four experiments were made with procain and we present an abbreviated protocol to illustrate the results.

Protocol 19. Cat, male, 2.94 kg.

9:51 a. m.: 0.06 mg. $\times$ kg. ouabain intravenously.

10:21 a. m.: 60.00 mg. $\times$ kg. procain intravenously in 20 per cent. solution. Prompt convulsion; respiration stopped in about one minute; heart feeble, but continued to beat. Artificial respiration started at once and continued for five minutes, during which the heart rapidly recovered. Complete recovery within fifteen minutes.

Although these first experiments were controlled by the administration of the same dose of procain to two normal cats with prompt death, in spite of artificial respiration the results were so striking that we undertook a more rigid control. For this purpose one of us injected four cats with either normal saline, or with 0.06 mg. $\times$ kg. of ouabain, and the other then administered 60 mg. $\times$ kg. of procain to each, carrying out artificial respiration in all, and was able to identify correctly the two cats which had received ouabain by the fact that they recovered while the other two died promptly.

Table 7 gives the important details of each of this series of twenty-three experiments and shows the administration of a dose of 0.02 mg. $\times$ kg. or over of ouabain effectively protects the heart from permanent stoppage by doses of the local anesthetics rang-

TABLE 7.—SHOWING EFFECT OF TOXICITY OF THE LOCAL ANESTHETICS ON THE PREVIOUS ADMINISTRATION OF OUBAIN, SUPPLEMENTED BY THE USE OF ARTIFICIAL RESPIRATION

Anesthetic	Dose		Dose of Ouabain		Artificial Respiration	Result
	Mg. $\times$ Kg.	% of Fatal	Mg. $\times$ Kg.	% of Fatal		
Apothesin.....	30.0	150	0.01	10	+	Death
	30.0	150	0.02	20	+	Death
	30.0	150	0.06	60	0	Recovery*
	40.0	200		..	+	Death†
	35.0	175	0.06	60	+	Recovery
Beta-eucain.....	18.7	150	0.06	60	+	Recovery*
Cocain.....	20.0	133	0.06	60	+	Death‡
	20.0	133	0.06	60	+	Recovery*
Holocain.....	12.5	125	0.06	60	0	Recovery*
Nirvanin.....	37.5	117	0.01	10	+	Death‡
	37.5	117	0.02	20	0	Recovery*
	40.0	125	0.06	60	0	Recovery*
Procain.....	60.0	142	0.02	20	+	Recovery
	60.0	142	0.06	60	0	Death
	60.0	142	0.06	60	+	Recovery
	60.0	142	0.06	60	+	Recovery
	60.0	142	0.06	60	+	Recovery
	60.0	142	0.06	60	+	Recovery§
	60.0	142	0.06	60	+	Recovery§
Stovain.....	37.5	135	0.02	20	+	Recovery
	37.5	135	0.06	60	+	Recovery
	40.0	148	0.06	60	+	Death!†
Tropacocain.....	25.0	125	0.05	50	+	Recovery

* In the experiments thus marked the heart did not stop.

† This animal had received the preceding dose of 30 mg. $\times$ kg. of apothesin 55 minutes earlier.

‡ In the experiments thus marked cardiac massage was employed.

§ In these two experiments the cats were identified as having received ouabain, unknown to the experimenter—see text.

¶ This animal had been given the dose of 40 mg. $\times$ kg. of nirvanin, recorded above, one hour earlier.

ing from 117 to 175 per cent. of the average fatal. Of the twenty-one experiments in which 0.02 mg. $\times$ kg. or more of ouabain was given, death followed the administration of the local anesthetic in only five. Of these five deaths, one followed the administration of twice the fatal dose of apothesin to a cat which, fifty-

five minutes before, had been given a dose of the same drug 50 per cent. greater than the average fatal and had recovered. A second death followed the administration of 148 per cent. of the fatal dose of stovain to a cat which had survived 125 per cent. of the average fatal dose of nirvanin given an hour earlier. Excluding these two, death followed the administration of these large doses of the local anesthetics, given after the previous administration of 20 to 60 per cent. of the fatal dose of ouabain, in only three out of nineteen cats. Artificial respiration was employed in all but five of the experiments, in four of which it was not necessary, while in one it was omitted purposely. Cardiac massage was tried in three of the experiments in which the heart did not show evidences of recovering, but it was without effect. By way of graphic illustration of this antagonism we introduce Figures 9 and 10, taken from two parallel experiments.

DISCUSSION

One fact of fundamental importance has emerged from the investigations detailed in the preceding pages; namely, that the "essential" elimination of all of the local anesthetics studied, excepting cocain and holocain, proceeds with great rapidity and is completed within a few minutes following the intravenous injection of a sublethal dose. The "essential" elimination of cocain and holocain is a much slower process, and in the case of cocain it may not be complete after periods of one to two, or more, days. The elimination of all of the local anesthetics in the cat has been shown to be accomplished by their destruction in the liver, as demonstrated by perfusion of that organ with solutions of the several drugs. In view of the close similarity of his behavior to toxic doses of the local anesthetics, it is highly probable that man also eliminates these drugs in the same way as the cat, especially since such rapid elimination by the kidney is most unlikely.

The local anesthetics can be divided into two groups according to their rates of destruction or "essential" elimination in the cat: Group 1, rapidly eliminated,

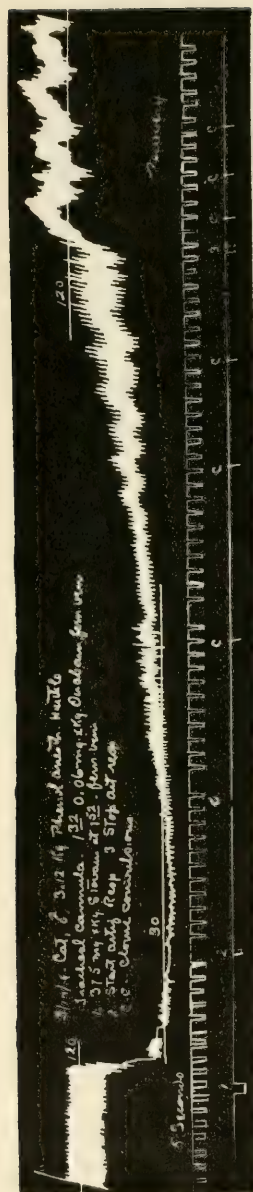


Fig. 9.

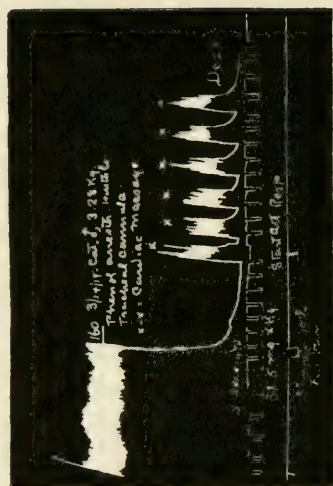


Fig. 10.

includes alypin, apothetin, beta-eucain, nirvanin, procain, stovain and tropacocain. Group 2, slowly eliminated, includes cocain and holocain. This grouping apparently applies also for man.

The prompt recovery of the cat following the intravenous injection of a just sublethal dose of any of the members of Group 1 is due to the rapid destruction of those drugs, while slower destruction in the case of cocain and of holocain explains the less rapid and less complete recovery after corresponding doses.

The ability of the cat to withstand repeated intravenous injections of large fractions of the minimal fatal vein doses of members of Group 1, when given at intervals of fifteen to twenty minutes, or to survive the slow and continuous injection of several times the average fatal vein dose, depends on the rapid "essential" elimination of those drugs, and the inability to withstand similar injections of corresponding amounts of the drugs of Group 2 is due to their slower elimination.

Rapid "essential" elimination of the members of Group 1 accounts for their relatively low toxicity by subcutaneous injection, or after their application to mucous membranes, in the cat and other animals, in spite of the fact that they are absorbed rapidly from the subcutaneous tissues and from some mucous surfaces. It also explains the marked reduction in the toxicity of these drugs effected by the delayed absorption produced by the simultaneous injection of epinephrin. Slower "essential" elimination of the members of Group 2 accounts for their relatively higher toxicity after subcutaneous injection, or application to mucous membranes, and for the lesser reduction in their toxicity effected by epinephrin.

Man also apparently recovers more rapidly from nonfatal acute poisoning by the members of Group 1 than from cocain, the only member of Group 2 on which we have data, and the difference is almost certainly again referable to his ability to destroy the drugs of the first group more rapidly and completely than he can cocain.

Finally, the successful resuscitation of cats, after intravenous doses of 117 to 200 per cent. of the average fatal, by means of artificial respiration and stimulation of the heart by epinephrin, and the protection of cats from death, following doses of 117, to 175 per cent. of the average fatal, by the previous stimulation of the heart by ouabain and the brief subsequent employment of artificial respiration, are both due primarily to the rapid destruction of the local anesthetics, by which the excess administered is promptly eliminated.

SUMMARY

1. The more important literature of acute intoxication in man from the local anesthetics is reviewed, and cases are cited to show the close similarity between the symptoms produced in man and in the lower animals, especially the cat.

2. The phenomena of acute intoxication in the cat, following the intravenous injection of the local anesthetics, are described, and the similarity of the phenomena produced by different members of the group is emphasized.

3. The maximum toxicity for each of the members of the series has been determined by rapid intravenous injection in the cat, and the relative toxicities of the several drugs are represented graphically.

4. The several different local anesthetics are shown to be mutually and quantitatively synergistic, so far as their fatal actions are concerned.

5. The capacity of the cat to withstand the intravenous injection of several times the fatal vein dose of any of the local anesthetics, except cocain and holocain, has been shown by repeated injections of large doses, or the continuous injection of relatively dilute solutions.

6. The toxicity of the local anesthetics for the cat, after subcutaneous injection, has been shown to depend on the ratio between the rate of absorption and that of elimination, and the local anesthetics can be divided into two classes with reference to that ratio. Five, or more than five, times the minimal fatal vein

dose of alypin, apothetin, beta-eucain, nirvanin, procain, stovain and tropacocain can be injected subcutaneously in the cat without causing death, while four, or less than four, times the fatal vein doses of cocain and holocain similarly injected prove fatal.

7. The simultaneous subcutaneous injection of epinephrin with the local anesthetics materially reduces the toxicity of the latter by delay in the rate of their absorption, but this reduction is much less marked in the cases of cocain and holocain than with the other members of the series, and is referable to their much slower "essential" elimination.

8. The absorption of several of the local anesthetics from the mucous membranes of the nose and pharynx of the cat has been shown to be no more rapid than from the subcutaneous tissues, and the urethra, bladder and vagina resist the absorption of these drugs to a great extent.

9. The elimination of the local anesthetics in the cat has been demonstrated to be due to their rapid destruction by the liver, and this takes place in the excised, perfused organ as well as in the liver of the intact animal.

10. Various efforts have been made to influence the toxicity of the local anesthetics for the cat, and severe acute hemorrhage and narcosis by hydrated chloral alone seem to have any material influence. Both of these measures tend to increase the cat's susceptibility to the toxic actions of the local anesthetics, probably by diminishing the rate of their destruction in the liver through impairment of the circulation.

11. All of the local anesthetics have been shown to be synergistic with epinephrin on the blood pressure in a manner analogous to cocain.

12. The employment of artificial respiration, combined with stimulation of the heart by the intravenous injection of epinephrin, is capable of saving cats from death following the intravenous injection of as much as twice the average fatal dose of the local anesthetics.

13. Stimulation of the heart by the previous injection of ouabain permits the cat to recover from intravenous injections of nearly twice the average fatal

dose of the local anesthetics, when the temporary paralysis of the respiratory center is combated by the use of artificial respiration.

14. The success of the last two measures depends on the rapid destruction of the local anesthetics by the liver.

IODIN TINCTURES, WATER SOLUBLE *

TORALD SOLLMANN, M.D.

CLEVELAND

(From The Journal A. M. A., Sept. 20, 1919, p. 899)

Proprietary preparations of iodine have been on the market for many years and advertised to physicians as superior to the official preparations—tincture of iodine and Lugol's solution (Liquor Iodi Comp., U. S. P.). The superiority claimed for these proprietary preparations is based on the allegation that the potassium iodide in the official preparations causes a local irritant action which is avoided in the proprietary preparations. The allegations seem improbable—certainly the local irritant action of potassium iodide must be regarded as negligible, as compared with that of hydriodic acid contained in the proprietary preparations. Rather, it was surmised that any difference in local irritation following the use of the proprietary preparations was due to the fact that the iodine content in these preparations was insufficient to produce the irritation or, on the other hand, sufficient to have only a slight, if any, therapeutic effect.

The manufacturers do not seem to have published, and presumably do not possess, any comparative data on the degree of irritation produced by their preparations, as compared with the official tincture of iodine after this has been diluted so as to reduce its iodine content to that of the proprietary preparations. It was therefore decided to determine this point by applying various iodine preparations of an equal iodine content to the skin and also by extending the experiments in other directions. In these experiments two widely

* From the Department of Pharmacology of the Western Reserve University School of Medicine.

* This investigation was supported by a grant from the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

advertised proprietary preparations—Burnham's Soluble Iodin¹ and Sharpe and Dohme's Surgodine²—were included.

Since these, however, are more or less secret in composition, I have devised a nonsecret preparation of the same character by the use of hydrogen iodid (hydriodic acid). The details of this preparation will be published in a pharmaceutical journal. Before use, each of these preparations was reduced by the addition of alcohol to a free iodin content of 2.2 per cent. (2.2 gm. in 100 c.c.).

The accompanying table shows the composition of the solutions used, the total iodin being the same, the principal difference being in the acidity, which ranges

COMPOSITION OF IODIN PREPARATIONS USED*

Nature of Preparation	Free Iodin	Percentage of Combined Iodin (Including HI)	Acidity as of HI.
Alcoholic solution	2.2	0.4	0.2
U. S. P. tincture (KI)	2.2	1.6	0.07
Alcohol with HI (author's formula) ..	2.2	0.7	0.6
Burnham's Soluble Iodine	2.2	2.7	1.4
Surgodine	2.2	1.2	0.8

* The numbers represent grams per hundred c.c. of preparation. Before use, each preparation was reduced with alcohol to a content of 2.2 per cent. of free iodin.

from a minimum of 0.07 per cent. in the U. S. P. tincture (containing potassium iodid) to a maximum of 1.4 per cent. in Surgodine. The special preparation which I devised is intermediate and contains 0.6 per cent. The total combined iodin (including potassium iodid or hydrogen iodid) ranges from 0.4 per cent. in the alcohol solution to 2.7 per cent. in the Burnham preparation.

1. Examination in the A. M. A. Chemical Laboratory indicated Burnham's Soluble Iodin to be a solution of iodin in alcohol made miscible with water by the presence of some iodid and containing approximately 3 gm. of free iodin and 2 gm. of combined iodin in 100 c.c. (J. A. M. A. **50**:1055 [March 28] 1908).

2. The A. M. A. Chemical Laboratory reported that Surgodine was an alcoholic liquid containing 2.51 gm. of free iodin and 1.78 gm. of combined iodin, probably present chiefly as hydrogen iodid, in 100 c.c. (J. A. M. A. **70**:257 [Jan. 26] 1918).

EFFECTS ON THE SKIN

The solutions were painted on the skin of the inner surface of the forearm, each solution covering an area of from 15 to 20 mm. diameter; each application was allowed to dry before the next was added.

The results did not show any significant or constant differences. The actual results indicated that Burnham's preparation was the more irritant, and the U. S. P. tincture (reduced to the same iodine percentage) was the less irritant, but the differences were so small that they could easily be accidental.

In a preliminary test with three applications, no material difference could be detected between the different solutions.

In a second series, a measured quantity of solution was applied to two spots in three courses. The first course consisted of five applications at intervals of five minutes, and then a pause of thirty-five minutes. At the end of this time, the skin of all the areas was slightly tender. The second course consisted of five applications during sixteen minutes, and then a fifty-five minute interval. There was now considerable tenderness, alike for all the areas. The third course comprised five applications during fifteen minutes. Forty minutes after the last application, all the areas were equally sore. The depth of the stains ran in decreasing order from Burnham's (most) through Surgodine, hydrogen iodide, U. S. P. tincture, and alcoholic (least); but the differences were not great.

After removing the excess of iodine with alcohol, the reddening and edema at this time appeared as follows: Burnham's most, distinct papular edema and reddening; then the alcoholic; then the others, which were about alike.

On the following morning, the order of irritation was as follows: most, both Burnham's areas; next, one of the alcoholic areas; next, one of the hydrogen iodide areas; next, Surgodine; least, U. S. P.

PRECIPITATION OF ALBUMIN

Irritation is often due to precipitation of proteins, and this probably applies to the irritant action of iodine. Therefore an investigation was made to determine whether there exist significant differences in this respect between the several iodine preparations.

Each of the diluted iodine preparations (2.2 per cent. iodine) was diluted with 10 volumes of water, to avoid precipitation of protein by the alcohol, and the diluted solution added to an equal volume of a 10 per cent. solution of natural egg-white in physiologic sodium chlorid solution.

The simple tincture, that with hydrogen iodid, Burnham's preparation, and Surgodine, produced an apparently identical coagulum. The U. S. P. tincture (diluted in the same way) produced only a turbidity; this also when the quantity of the iodine solution was doubled. Next morning, the albumin was coagulated as with the others. The reaction to litmus remained neutral.

The restraining action of the potassium iodid is limited, for the addition of an equal quantity of the potassium iodid tincture to the hydriodic acid tincture does not prevent immediate coagulation (1 volume potassium iodid tincture, 1 volume hydrogen iodid tincture, 10 volumes of water).

It is evident, therefore, that the potassium iodid of the official tincture has a restraining effect on the coagulation of protein. This would tend to make it less irritant than the other preparations. It is conceivable that this may be disadvantageous in skin protection, in which the fixative action is probably desirable. In this case, however, there is no object in using a water-miscible preparation, and the simple alcoholic solution of iodine would be fully as good as any of the others.

CAPILLARITY AND SPREADING

Dilute tinctures of iodine, when placed on the skin or on parchment, tend to distribute themselves so that the solution becomes most concentrated at the periphery (as in the accompanying illustration). In this manner, the periphery of an application on the skin may be blistered when the center is scarcely affected. If this property were more marked in one specimen than another, this would be equivalent to greater irritation. The illustration shows that this uneven distribution

runs in the order: U. S. P. (potassium iodid tincture), most; Burnham's soluble iodine; hydrogen iodid tincture; Surgodine; alcoholic tincture (containing no potassium iodid), least.

The differences are in favor of the simple alcoholic tincture (which was formerly official) and adverse to the tincture containing potassium iodid (which is now official); but they do not impress me as of great importance, since they are even less marked on the skin.

I tried also to demonstrate differences in capillarity by the spreading or rise through filter paper; but the variations were insignificant.

CONCLUSIONS

The presence of potassium iodid in the official tincture of iodine does not seem to render this preparation more irritant. On the contrary, it is somewhat less irritant to the skin, and much less precipitant to proteins, than the simple alcoholic tincture, or the secret and nonsecret "miscible tinctures." The more even spreading and the more rapid coagulation of protein render the simple alcoholic solution of iodine probably the best for the "disinfection" of the skin; while the delayed protein precipitation by the U. S. P. tincture would probably render this somewhat superior for the disinfection of open wounds. The secret and non-secret "water-soluble tinctures" do not appear to have any material advantages.

LOCAL ANESTHETICS: DO THEY PRECIPITATE PROTEINS?*

TORALD SOLLMANN

CLEVELAND

(From *The Journal of Pharmacology and Experimental Therapeutics*,
October, 1919, p. 135)

Precipitation of proteins would be an important property for local anesthetics, in that it might lead either to a desirable astringent action, or to undesirable irritation. The following experiments show, however, that most of the anesthetics now available do not precipitate proteins, and none did so to a serious degree.

Method.—The aqueous solution of the anesthetic (2.5 c.c.) was poured on 5 c.c. of the protein solution. This was done slowly, with a view to possible ring tests, but none occurred. The solutions were then shaken.

The following filtered protein solutions in 0.9 per cent. NaCl were used: "Albumen" = a 10 per cent. solution of moist egg white. "Serum" = 1 per cent. of dried serum. "Peptone" = 1 per cent. of Witte's peptone.

The experiments were made by Mr. O. H. Schettler. The results may be grouped as follows:

A. Saturated solutions of "insoluble" anesthetics. These were made from 1 per cent suspensions.

Anesthsin, cycloform, orthoform-new, propaesin. No precipitate with any of the proteins.

B. 1 per cent solutions of soluble anesthetics.

Alypin (hydrochlorid), cocain (hydrochlorid), holocain (hydrochlorid), procain (hydrochlorid). No precipitate with any of the proteins.

Apothesin (hydrochlorid): Precipitated with albumen, not with serum or peptone.

* From the Pharmacological Laboratory of the Medical School of Western Reserve University, Cleveland.

* Received for publication July 30, 1919.

C. Five per cent. solutions of soluble anesthetics.

Alypin (hydrochlorid), cocain (hydrochlorid), procain (hydrochlorid). No precipitate with any of the proteins.

Apothesin hydrochlorid: Turbidity with albumen and opalescence with serum; no change with peptone. The precipitates do not clear on acidulation with HCl.

Five per cent. acidulated solutions of the "insoluble" anesthetics. The anesthetics were brought into solution by a minimum of diluted HCl.

Cycloform, orthoform-new, propaesin. These do not precipitate any of the proteins. Precipitation does occur when the solutions are mixed, but it redissolves on the addition of acid, and is therefore due to the precipitation of the anesthetics by the alkalinity of the proteins.

Anesthesin: Turbidity (not cleared by acid) with serum. No precipitation of albumen or peptone.

It may be remarked that the neutral and acid *orthoform* solutions turned yellow on standing; the color deepening to reddish brown on acidulation with hydrochloric acid.

THE ROLE OF THE BROMID SALTS ON RHYTHMICALLY CON- TRACTING ORGANS

I. THE ACTION OF THE BROMIDS ON THE ISOLATED MAMMALIAN HEART *

THEOPHILE KRUSE

*(From The Journal of Pharmacology and Experimental Therapeutics,
October, 1919, p. 137)*

Although the general bromid reactions on the heart are now fairly well understood, less seems to be known of their detailed behavior, such as may be observed on the isolated mammalian heart. In a previous report the author with Prof. C. W. Greene¹ showed that a perfused frog heart is slightly augmented in rate and later slightly decreased by a bromid substituted Ringer's solution. Such a Ringer's solution is just as effective as the ordinary chlorid Ringer's solution for maintaining the rhythmicity of the frog heart. The following experiments were performed to determine whether the isolated mammalian heart behaved in a similar manner.

The older literature, as reviewed by Krosz² shows that the heart is depressed by the bromids. These reports are not entirely trustworthy in interpretation since they are essentially based on results obtained by applying concentrated potassium bromid solutions on the surface of the heart. By such a method the

* The facts presented in this paper were used as a part of a thesis rendered for the degree of Doctor of Philosophy, at the University of Missouri, under the direction and advice of Prof. C. W. Greene to whom I express my gratitude. This investigation was partly supported in 1914 by a grant from the Committee on Research of the Council on Pharmacy and Chemistry of the American Medical Association.

* From the Department of Physiology and Pharmacology, Laboratory of Pharmacology, University of Missouri.

* Received for publication July 10, 1919.

1. Greene and Kruse: *J. A. M. A.* **61**: 271, 1913.

2. Krosz: *Arch. exp. Path. Pharm.* **6**: 1, 1876. (Old literature.)

typical bromid reaction is obscured by the depressant potassium action and by osmotic effects. Other reports are based on clinical cases which obviously are obscured by the bromid reaction on the central nervous system. Although Krosz attributed the depression of the frog heart to the potassium factor he believed nevertheless that much was due to the bromid factor.

In recent years, it has been established by Loeb on marine animals ³ and by Lingle,⁴ Benedict ⁵ and others that the bromids are stimulating on the rhythmic muscular tissues, such as the turtle heart if the sodium salt is used in isotonic concentrations. In a previous report ⁶ I have indicated that smooth muscle is similarly stimulated. In the skeletal muscle of the frog, Loeb ⁷ has observed stimulation by the bromids. But in the perfused rabbit heart Busquet and Pachon ⁸ have found sodium bromid nontoxic.

METHOD

The heart of a cat or a dog was excised in the usual manner and was suspended in a perfusing apparatus designed in this laboratory. The coronaries were perfused with a Ringer's solution and was compared with a similar solution in which the chlorid salts were replaced by the corresponding bromid salts in equimolecular concentrations. Both the chlorid and the bromid solutions were diluted with corresponding quantities of defibrinated blood taken from the same animal.

The usual plan of experiment was to establish rhythmicity by the chlorid solution and then to test the bromid reaction by perfusion for short intervals, usually three minutes, and then allowing the heart to recover by the chlorid perfusion.

3. Loeb: *Am. J. Physiol.* **3**: 327, 1900.

4. Lingle: *Am. J. Physiol.* **4**: 272, 1901.

5. Benedict: *Am. J. Physiol.* **13**: 192, 1905.

6. Kruse: *Am. J. Physiol.* **42**: 595, 1917.

7. Loeb: *Fick's Festschrift*, Braunschweig, 1899.

8. Busquet and Pachon: *J. de Physiol.* **11**: 243, 1909.

EXPERIMENTAL RESULTS

1. THE EFFECT OF THE BROMID PERFUSION ON THE ISOLATED MAMMALIAN HEART

Perfusion of the isolated mammalian heart by the bromid substituted Locke's or Ringer's solution produces at first an increase in the rate and the amplitude of the contractions and is followed within one to three minutes by a decrease. The increase in the amplitude falls on both the systole and the diastole of the contraction, Figures 1 and 3. During the period of depression the perfusion rate is greatly decreased due to the constriction of the coronaries.

In some experiments after a certain depressive effect was reached there occurred a marked reaction at the instant when the perfusion was changed from the bromid solution to the chlorid solution. This was manifested by a sudden decrease in the rate and the amplitude of the contraction greater than would have occurred had the perfusion not been changed (Fig. 3). Sometimes this reaction did not take place, particularly when the bromid solution was removed before depression had set in. The typical response is illustrated in Figure 2 in which an experiment is plotted and in Figure 3 in which the figures written in at the top of the trace designate the rate per minute and amplitude in millimeters, respectively. These show that the rate and the amplitude of the heart increased at once during the bromid perfusion and passed quickly into a state of depression in which the amplitude decreased while the rate was still increasing.

The secondary depression may be explained in part by the constriction of the coronaries, since an increase in the perfusion pressure retards the depression for a short time. Soon after the appearance of the secondary depression, arrhythmia occurs which can be removed for a time by a similar increase in the perfusion pressure, but the coronaries tend to constrict still more and the same depression is repeated (Fig. 4).

In three experiments the heart was revived from the start by a bromid Ringer's solution. One of these experiments is represented by a graph in Figure 4.

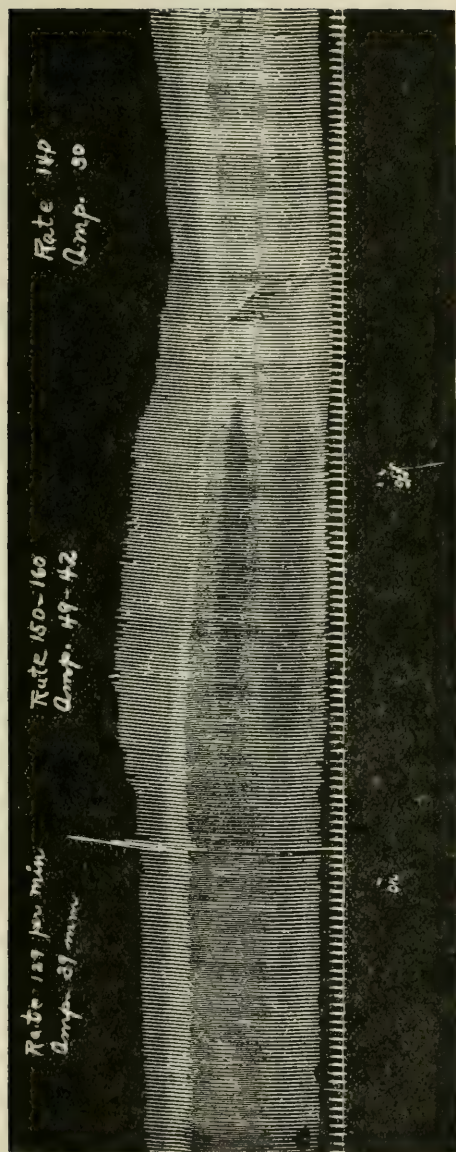


Fig. 1.—Bromid Locke perfusion of the isolated heart of a puppy. This shows a mild effect of a bromid Locke's perfusion. Time in seconds.

It shows the decreased heart activity being interrupted after each increase of perfusion pressure. Coincident with the decrease in activity there occurs a decrease of the perfusion rate. These hearts remained rhythmic from ten to eighteen minutes. Control experiments with a chlorid Ringer's solution maintain hearts in rhythm for several hours.

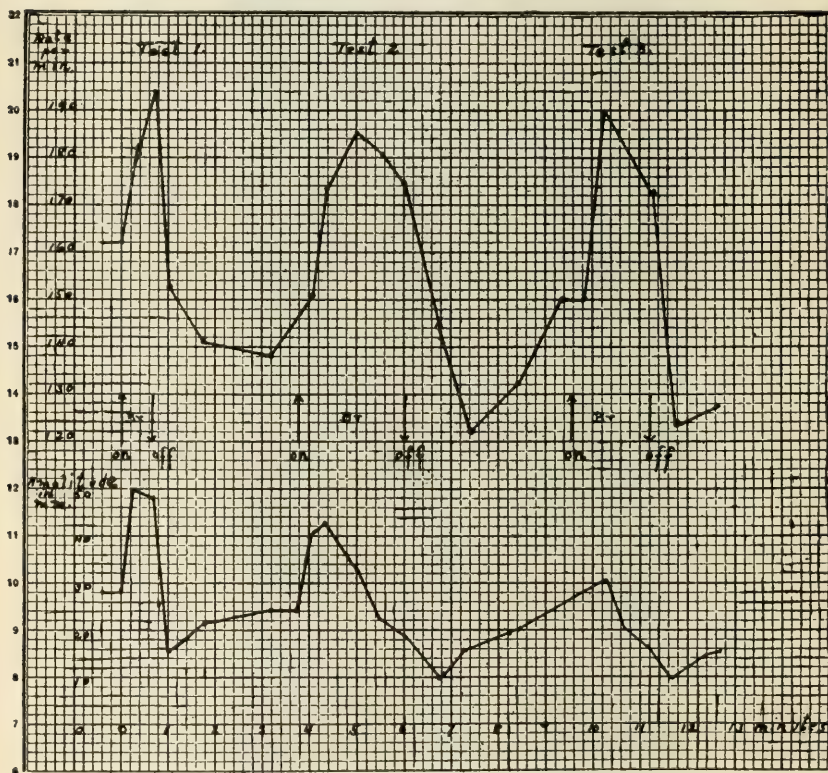


Fig. 2.—A graph representing alternate perfusion of a chlorid and a bromid Locke's solution on the isolated heart of a puppy. This shows the marked increase in the rate and the amplitude during a bromid perfusion and the secondary decrease following it. Note that the amplitude decreases while the rate is still increasing. The space between the arrows "on" and "off" indicates the bromid perfusion.

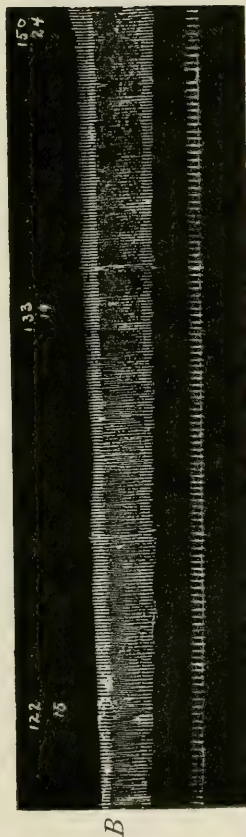
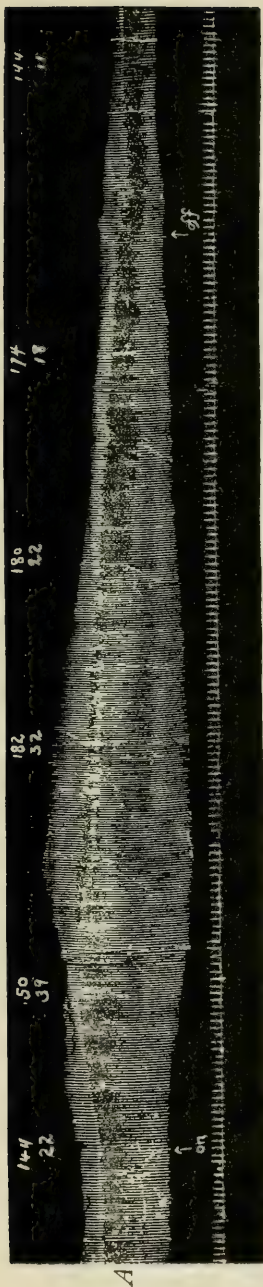


Fig. 3.—Bromid Locke perfusion of the isolated heart of a puppy. *B* shows the recovery of *A*. Note the secondary decrease after the primary increase in both rate and amplitude. Also note the sudden decrease after the bromid solution is replaced by the chlorid solution. Time in seconds.

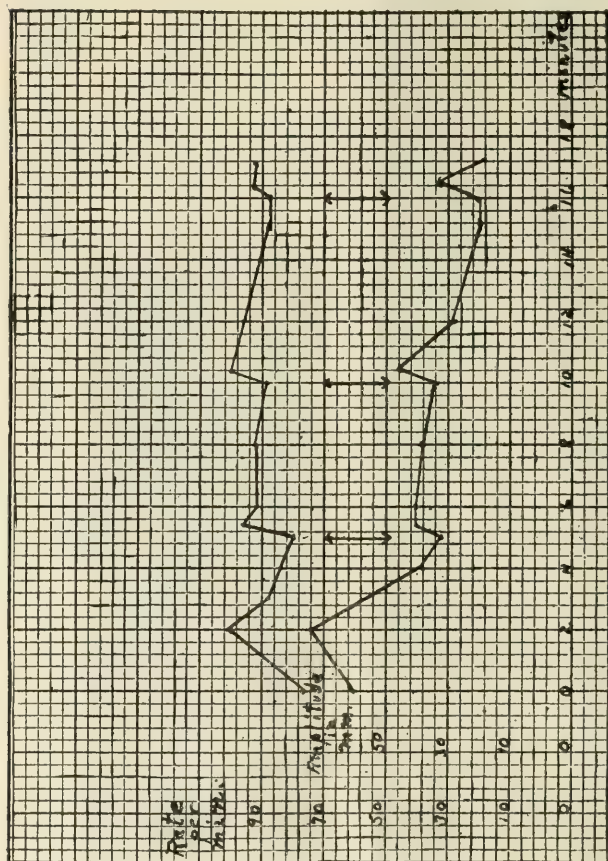


Fig. 4.—A graph representing a constant perfusion of a bromid Ringer's solution on a cat's heart. This shows the eventual decrease after the primary augmentation and the beneficial influence of increasing the perfusion pressure (arrows).

2. UNIFORMITY OF RESULTS

To show the relative uniformity of the results all of the tests are summarized according to the effect, shown in Table 1. Those experiments not showing

TABLE 1.—SHOWING THE FREQUENCY OF CHANGE IN THE RATE AND THE AMPLITUDE OF THE CONTRACTIONS IN DOG AND CAT HEARTS CALCULATED FOR ALL EXPERIMENTS

	Primary Increase, per Cent.	No Change, per Cent.	Primary Decrease, per Cent.	Eventual Decrease, per Cent.
Dog (including 35 tests)				
Rate.....	85.7	5.7	8.6	65.0
Amplitude.....	68.6	8.6	22.8	90.0
Cat (including 25 tests)				
Rate.....	39.0	9.0	52.0	80.0
Amplitude.....	36.0	14.0	50.0	79.0

depressive effects were due to the shortness of the test. In the cat heart the early stimulation was very short in duration and was absent in many tests. When a heart was tested repeatedly with a bromid Ringer's solution it was found that the later tests were less intense (Fig. 5). This change in the behavior may be the manifestation of a surface action caused by the gradual substitution of the chlorids in the heart muscle in contrast to the sudden substitution occurring in the earlier tests.

DISCUSSION

The constriction of the coronaries seems to be the principal cause for the depressive effect of a bromid perfusion through the mammalian heart. This constriction need not be looked on as the initiation of a new process but is an acceleration of a process taking place in all perfused hearts. Cushny and Gunn⁹ described such an effect on the coronaries by blood serum or plasma. They attributed this effect as due to the alteration of the heart in the isolated condition. Morawitz and Zahn¹⁰ and Hermel¹¹ found that old

9. Cushny and Gunn: *J. Pharm. and Exper. Therap.* **5**: 1, 1913.

10. Morawitz and Zahn: *Arch. klin. Med.* **116**: 364, 1914.

11. Hermel: *Arch. klin. Med.* **115**: 207, 1914.

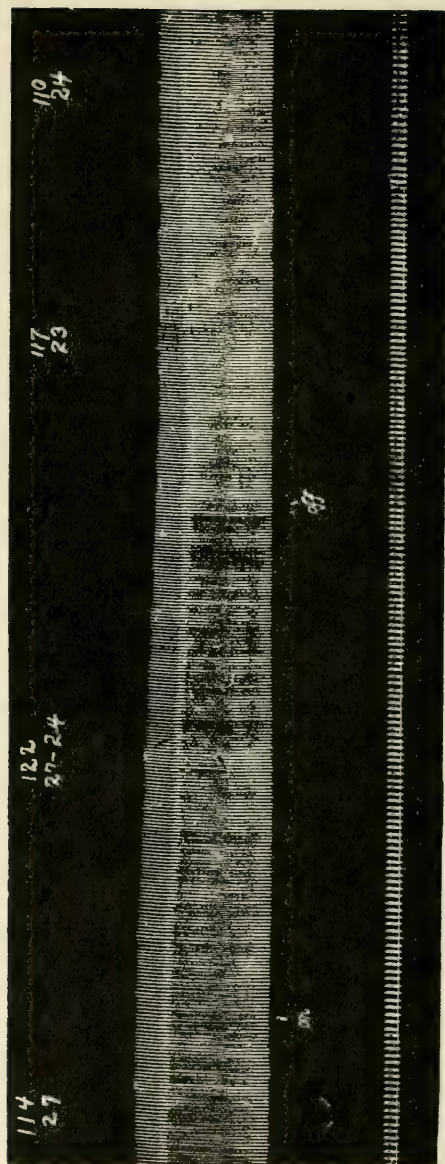


Fig. 5.—Bromid Locke perfusion of the isolated heart of a puppy. Showing that later perfusion tests act less intensely. Note the rate is increased, but the amplitude is reduced. (Same heart and solution as in Figure 3.)

perfused blood and standing defibrinated blood have this constrictor influence on the coronaries when the heart is perfused. Hermel believed that a new colloidal arrangement of the plasma was a possible explanation.

In later experiments¹² it was found that smooth muscle showed an increase in tonicity when suspended in a bromid Ringer's solution. By direct observation on the coronary outflow and by analogy on other smooth muscle tissues it was concluded that the stimulation of the smooth muscle of the coronaries leads to a depression of the heart through asphyxiation. This view does not exclude the possibility of a later depression by action directly on the heart muscle by the bromid ions.

The early stimulation of the heart muscle seems to be the characteristic reaction typical for most motor types of protoplasm, since all three forms of muscular tissues are similarly affected. In the cold-blooded animals the depression is less intense than in the mammalian species possibly because the former type of animals thrive on a greater economy of oxygen. Recently Macht and Hooker¹³ have shown that perfusion of the medulla of a mammal by a bromid solution stimulates the respiratory and cardio-inhibitory center. It is therefore evident that the bromids are more or less irritating to most tissues of the body. Only later do symptoms of depression occur caused perhaps by a disturbance of the oxidative mechanism. It is noteworthy in this connection that the central nervous system of the frog is not depressed by sodium bromid.

The augmentation of the heart by the bromids in the intact animal is not pronounced for several reasons. First, the concentration of the bromids used in our experiments is greater than can be realized in the blood of the intact animal, since isotonic concentrations were used. Second, the early action of the bromids on the cardio-inhibitory center counteracts the stimulation of the heart. Third, isolation of the heart produces changes in the heart probably due to the early

12. Kruse: Footnote 6.

13. Macht and Hooker: *J. Pharm. and Exper. Therap.* **11**: 63, 1918.

asphyxiation so that this influence is more prominent in the isolated condition. Such an alteration has been indicated by Guthrie and Pike ¹⁴ who showed that the isolated heart responds readily to pressure changes whereas the heart *in situ* fails to do so even after all extrinsic nerves are cut.

SUMMARY

1. The action of the bromids on the perfused isolated mammalian heart is characterized by a primary increase followed by a decrease in the rate and the amplitude of the contractions.

2. The depression occurs more quickly on the amplitude than on the rate.

3. The secondary depression is looked on as being due to the constriction of the coronaries. This view does not exclude the possibility of a direct influence of the bromid ion.

4. The withdrawal of the bromid perfusion from the heart leads to a sudden depression greater than would have occurred if the bromid perfusion had been continued.

5. A bromid Ringer's or Locke's solution is inadequate to maintain rhythmicity in a perfused isolated mammalian heart for as long a time as a similar chlorid solution. In the frog heart, however, the bromid Ringer's solution is at least as efficient as a chlorid Ringer's solution.

14. Guthrie and Pike: Am. J. Physiol. **18**: 14, 1907.

II. THE ACTION OF THE BROMIDS ON SMOOTH MUSCLE*

THEOPHILE KRUSE

(From *The Journal of Pharmacology and Experimental Therapeutics*,
October, 1919, p. 149)

In a previous communication,¹ the author showed that the coronaries of the heart are constricted by a perfusion of a bromid Ringer's solution. The following experiments were performed to determine whether other types of smooth muscle show augmented activity under bromid treatment.

Stiles² showed that isolated esophagus strips of the frog are maintained in rhythm by a bromid Ringer's solution. Prochnow³ showed that the sodium halides increase the tonus of the uterus and the smooth muscle of arteries in the order of fluorids, iodids and bromids. The rate and power of the contraction were not found affected, but the excitability was found increased. Very dilute solutions were used. Winkler⁴ showed that sodium bromid like sodium chlorid at first increases the irritability of the frog stomach and later decreases it. He used twentieth-normal and tenth-normal sodium bromid and sodium chlorid solutions in the pure form.

METHOD

Smooth muscle strips or segments taken from the intestine of a cat or dog were suspended in an oxygenated chlorid Ringer's solution at 38 C., accord-

* A preliminary report of this investigation was read before the American Physiological Society, December, 1916. *Am. J. Physiol.* **42**: No. 4, 1917. The facts presented in this paper were used as a part of a thesis rendered for the degree of Doctor of Philosophy, at the University of Missouri, under the direction and advice of Prof. C. W. Greene to whom I express my gratitude.

* From the Department of Physiology and Pharmacology, Laboratory of Pharmacology, University of Missouri.

* Received for publication July 10, 1919.

1. Kruse: *J. Pharmacol. and Exper. Therap.* **14**: 63, 1919.

2. Stiles: *Am. J. Physiol.* **4**: 272, 1901.

3. Prochnow: *Arch. internat. de Pharmacol. et de Therap.* **21**: 287,

4. Winkler: *Pflueger's Arch.* **71**: 357, 1898.

ing to the method of Magnus.⁵ After rhythmicity was established the tissue was tested with a bromid Ringer's solution containing equimolecular quantities of bromids for the chlorids. Sometimes two segments were taken from adjacent portions of the intestine and were mounted, one in a chlorid and the other in a bromid Ringer's solution. After variable intervals these solutions were interchanged, thus controlling the activity of a strip with another as well as controlling identical solutions with more than one strip.

The test solutions were always tested by the freezing point method for its molecular concentration and by Mohr's method for its halide concentration. The tissues were taken principally from the intestines of cats and dogs. Some tests were made on the fallopian tubes of the dog, the esophagus, stomach and intestines of the frog, and esophagus, stomach and oviduct of the turtle. If the organ was small it was mounted as a segment in a longitudinal manner. Larger organs were cut into narrow strips or circular segments and mounted as such.

RESULTS

Intestinal segments suspended in a bromid Ringer's solution show an augmentation in rate, amplitude and tonicity. Changes in rate and amplitude were variable, but changes in tone were nearly always observed.

Rhythmicity.—When two strips or segments of intestine were mounted at the same time, one in a chlorid and the other in a bromid Ringer's solution, the bromid strip was the first to initiate rhythmicity. If the solutions were then interchanged the inactive strip became active and the active strip became inactive or showed decreased activity (Fig. 1). This reaction was repeated a number of times.

Sometimes an arrhythmic segment became perfectly rhythmic in the bromid Ringer's solution (Fig. 2). Since solutions containing a small quantity of blood showed better rhythmicity in both halide solutions, the regularizing influence of the bromid solution was asso-

5. Magnus: Pflueger's Arch. **102**: 123, 1904.

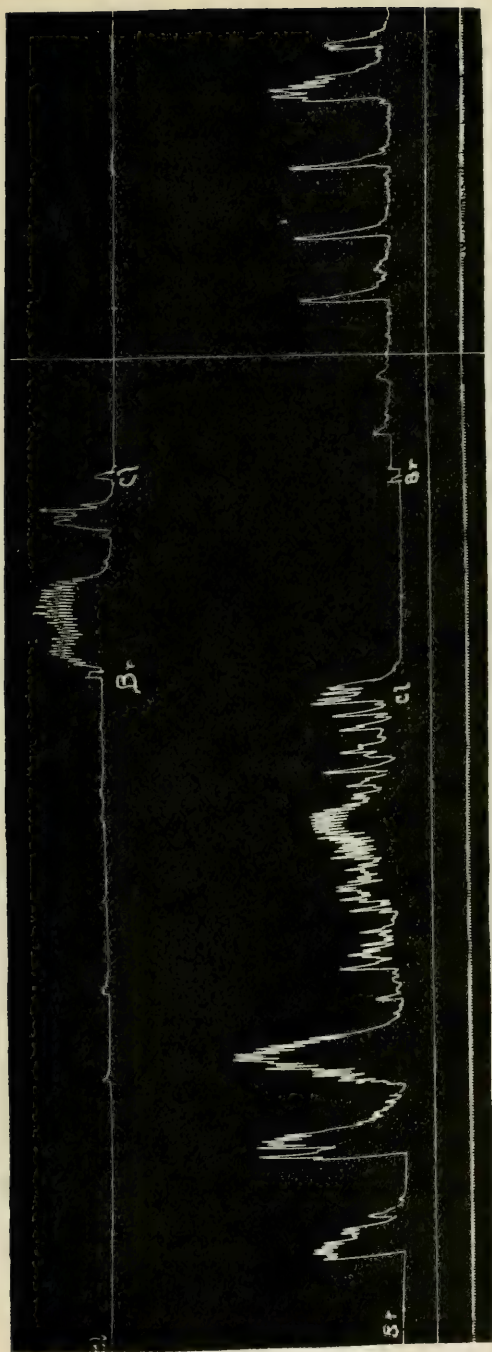


Fig. 1.—Two segments of cat intestine. There is an interval of thirty seconds between the two figures. The top segment was mounted in a chlorid Ringer's solution and the lower segment in a bromid Ringer's solution. The solutions were interchanged at the marks in the third line. The bromid solution caused an augmentation in each case. Temperature 38 C. Time in five seconds.

ciated with more perfect tissue respiration brought about either by an increased activity of the oxidative enzymes or by a facilitation in the oxidative mechanism. Later experiments showed that catalase activity is increased by the bromids.

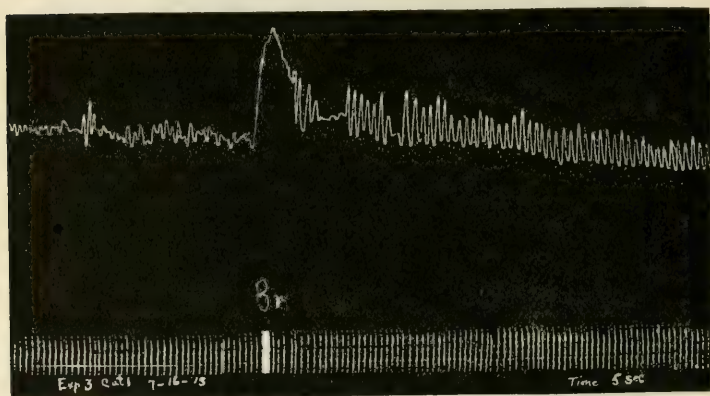


Fig. 2.—Intestine of cat, showing the regularizing influence of the bromid solution. The segment was immersed in a bromid Ringer's solution at the mark. Note the primary increase of tone. Temperature 38 C. Time in five seconds.

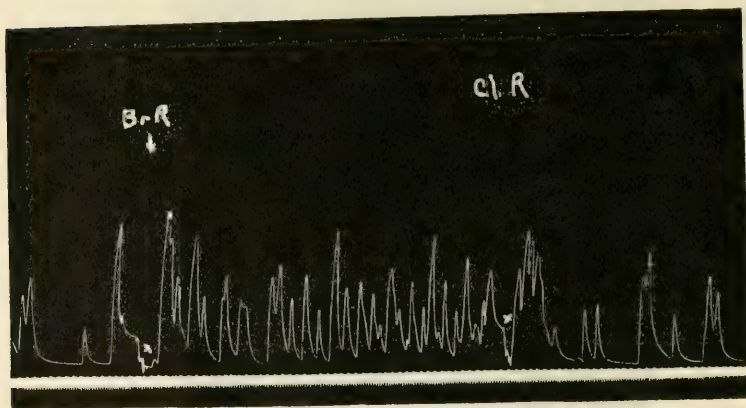


Fig. 3.—Intestine of cat, showing the stimulating influence of a bromid Ringer's solution. Note the reversibility of the reaction. Temperature 37 C. Time in two and one-half seconds.

The increase in the rate was as high as 20 per cent. in many experiments, but was very slight when the preexisting rhythm was regular. The marked increases of rate were always associated with an irregular pre-existing rhythm (Fig. 3).

Amplitude.—Changes in the amplitude were inconstant and slight. A sluggishly acting intestine showed the more marked increases in amplitude. Very active

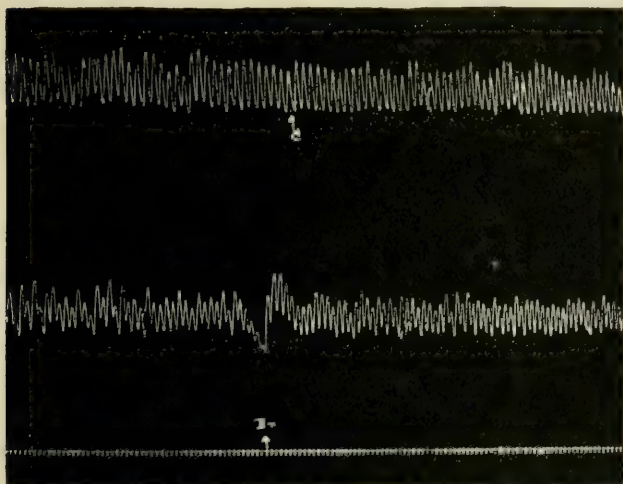


Fig. 4.—Two segments of cat intestine, showing that the augmentation of a bromid solution is less intense if the segment is rhythmic. The top segment is in bromid and the lower segment in chlorid Ringer's solution. At the mark the solutions are interchanged. Note the augmentation of rate produced in the bromid solution. Time in five seconds.

intestinal segments showed practically no further changes in amplitude. If the tonicity change was very great the amplitude was sometimes decreased. The most marked increases in amplitude were associated with moderate tone changes.

Tone.—An arrhythmic segment responded by a marked increase of tone in the bromid solution. A high degree of tone was rarely long maintained. It

was usually followed by a decrease simulating fatigue. Sometimes it really decreased below the previous chlorid level. If the tissue was immersed at this stage in chlorid solution, the tone decreased to a still lower level showing that the tissue was still augmented in the bromid solution. Sometimes the secondary decrease was absent. Such cases, however, were the exception.

A very rhythmic segment showed occasionally only slight change in tone and indeed, sometimes was just perceptible (Fig. 4). The response was in all cases dependent on the previous activity of the tissue.

THE EFFECT OF THE BROMIDS ON OTHER SMOOTH MUSCLE TISSUES

Fallopian Tube of the Dog.—The fallopian tube of the dog subjected to a bromid Ringer's solution responded essentially as the intestine of the cat and dog with the exception that the tone was better maintained during the primary rise.

Smooth Muscle of the Frog.—The results on the esophagus, stomach and intestine of the frog were inconstant. It was indeed difficult to detect changes when these segments were changed from the chlorid to the bromid Ringer's solution. In exceptional cases augmentation was observed.

Smooth Muscle of the Turtle.—The esophagus, stomach and intestine of the turtle seemed little affected by the bromid solution just as it was in the frog. The oviduct of the turtle simulated the results found characteristic in the mammalian intestine but were less intense.

Point of Action.—Plexus free preparations were made according to the method of Magnus,⁶ with and without the application of atropin to determine whether the bromid augmentation was due to a stimulation of either muscle or nerve elements or due to a stimula-

6. Magnus: Pflueger's Arch. **102**: 349, 1904.

tion of both. Augmentation was observed in plexus free preparations with atropin, but was of less magnitude than when the plexus was intact. The toxic effect of atropin on the muscle is probably in part responsible for the decrease. The conclusion was reached that both muscle and nerve are stimulated by the bromids in plexus containing organs. Prochnow⁷ concluded that the bromids stimulate smooth muscle directly on the basis of experiments on the carotid artery of ox. Such preparations are reported to be free from intrinsic nerve cells.

DISCUSSION

In a previous communication it was shown that the bromids act as mild stimulants to skeletal muscle, cardiac and smooth muscle of the heart and to certain medullary nerve centers.⁸ In this paper it is shown that many types of smooth muscle are stimulated. The early effect of the bromids therefore appears to be a primary stimulation.

Symptoms of stimulation have been observed clinically⁹ both on the central nervous system and on the gastro-intestinal tract. Depression is a later manifestation.

The variable quantitative changes occurring in the tissue are dependent on its physiologic condition. In the strip technic the tissue goes through variable degrees of asphyxiation. A mild stimulant has accordingly a profound effect. The same principle is evident in fatigued muscle nerve preparations of a frog. It is for this reason that segments contracting arrhythmically show the most profound changes whereas the changes are less intense in rhythmic preparations.

7. Prochnow: *Arch. internat. de Pharmacol. et de Therap.* **21**: 287, 1911.

8. Kruse: *J. Pharmacol. and Exper. Therap.* **14**: 63, 1919.

9. Fere: *Nouvelle Iconographie de la Salpêtrière* **15**: 435, 1902.
Mitchel: *Therap. Wehnschr.* **3**: 821, 1896. Hodges: *South Med. Record*, Atlanta, 1896, 505.

SUMMARY

1. The bromids stimulate smooth muscle slightly when the tissue is in a normal and rhythmic condition and markedly when it is arrhythmic or in a partly fatigued state.

2. The primary stimulation of the bromids seems to be a property common for many tissues.

THE PLASMA AND BLOOD CLOTTING EFFICIENCY OF THROMBOPLASTIC AGENTS IN VITRO AND THEIR STABILITY *

P. J. HANZLIK

AND

C. M. WEIDENTHAL

CLEVELAND

(From The Journal of Pharmacology and Experimental Therapeutics,
October, 1919, p. 157)

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I. INTRODUCTION

It was previously shown ¹ that the entire group of hemostatic agents is rather limited in their usefulness, due in part to the difficulty of applying crucial tests in determining their efficiency under actual conditions

* From the Pharmacological Laboratory, School of Medicine, Western Reserve University, Cleveland.

* Received for publication July 24, 1919.

* This research was supported in part by a grant from the Therapeutic Research Committee, Council of Pharmacy and Chemistry of the American Medical Association.

1. Hanzlik: J. Pharmacol. & Exper. Therap. **10**: 523, 1918; **12**: 71, 1918; **12**: 119, 1918.

of wound hemorrhage. This is particularly true of the thromboplastic type of agents derived from tissues or blood. Among these are kephalin, and such commercial preparations as thromboplastin, coagulen, hemostatic serum and coagulose. The claims made for the hemostatic qualities of these appear exaggerated when it is borne in mind that hemorrhages of whatever origin are most capricious, and generally tend to cease spontaneously, even in hemophilia.

There is no doubt that kephalin, at least, accelerates the clotting of oxalate and peptone plasma in the presence of fresh serum *in vitro*, and it is largely on this basis that its use in hemorrhages has been advocated, although, of course, this does not necessarily follow. Lack of success with these agents has been attributed to the use of old and deteriorated products and it is conceivable also that their activity varies with the dosage and concentration.

It is the object of this communication to compare the thromboplastic activity of several different products; the activity of fresh and old preparations, and the relation of concentration to their activity *in vitro*. Subsequent communications will deal with hemostatic properties in different kinds of hemorrhages in animals, and toxicity.

The thromboplastic agents tested were secured from various sources on the market, from the manufacturers direct, and two preparations of kephalin were made in the laboratory. These may now be described.

1. DESCRIPTION OF THE THROMBOPLASTIC AGENTS TESTED

Most of the commercial thromboplastic preparations are not secret or patented remedies, although the chemical composition of some, particularly as to active constituents, is vague or unknown. These have been evolved or inspired by reputable investigators in connection with studies on the coagulation of blood. For convenience, the various agents may be conveniently classified into three groups.

Other theories of blood coagulation, notably those of Morawitz and Hammarsten,⁵ are based on different conceptions. However, it is not the object of this paper to discuss the theories of blood coagulation. For these the current textbooks of physiology and works of the respective investigators may be consulted. The chemical and physical properties of kephalin are described in monographs by Bang³ and Hugh MacLean.⁶ It should be borne in mind that kephalin is insoluble in water, but swells like lecithin, forming an emulsion or colloidal suspension, which is modified by various factors. Owing to this the kephalin content of aqueous (normal saline) extracts of tissues must be variable and uncertain.

3. *Serum Products*.—To this class belong preparations known as coagulen, hemostatic serum and coagulose. These are prepared from serums of different animals, usually, from horse serum, although rabbit and human serums have also been used. The exact composition of these agents is unknown.

(a) Hemostatic serum: This is alleged to be a serum derivative discovered by Dr. Vincent A. Lapenta,⁷ composed principally of prothrombin and antiprothrombin in physiologic balanced solution. The circulars of the manufacturer make extravagant claims for this alleged hemostatic in "pulmonary hemorrhage, purpura hemorrhagica, intestinal bleeding, bleeding of the new-born, indolent ulcers, and hemorrhages incidental to various surgical procedures such as bone operations, intracranial surgery, herniotomy, tonsillectomy, amputations and hysterectomy." The experimental evidence submitted by Lapenta is uncritical and unsatisfactory.

(b) Coagulen: This preparation was inspired by Kocher and Fonio⁸ of Berne, and is alleged to be a "physiologic styptic prepared from the natural coagu-

5. Hammarsten-Hedin: Text Book of Physiological Chemistry, 1915, 7th ed., Wiley & Sons.

6. MacLean, Hugh: Lecithin and Allied Substances, 1918, Longmans, Green and Company.

7. Lapenta, V. A.: Therapeutic Gazette **34**: 23, 1918.

8. Fonio: Corr.-Blatt f. Schweiz. Aerzte **43**: 385, 422, 456, 1913.

lents of animal blood contained in the blood platelets. It has the characteristics of a lipoid." If kephalin is meant, it is difficult to understand why platelets should be selected in preference to other abundantly supplied organs such as brain. The preparation is marketed in three forms: (1) as dry powder containing lactose; (2) as 3 per cent. sterile solution in ampules, and (3) tablets. The object of the lactose is stated to be for facilitating solution in water.

Coagulen is recommended for use by practically all known methods of administration and for all kinds of hemorrhages irrespective of cause, origin or etiology, with sweeping generalizations giving the impression of a panacea for bleeding ills. The same may be said of hemostatic serum and coagulose.

(c) Coagulose: This is claimed to be an acetone-ether precipitation product of normal blood serum (presumably horse serum) inspired and originated by G. H. A. Clowes and F. C. Busch⁹ and marketed under the name of "Coagulose" by Parke, Davis & Co. "It is a sterile, soluble, anhydrous powder, containing the fibrin ferment necessary for clotting blood. It is readily soluble in cold water and concentrations two or three times that of original serum concentrations . . . retaining its active principles unimpaired for long periods of time." The experimental and clinical evidences have been mainly supplied by Clowes and Busch. According to them "Practical experience would indicate that in the large majority of cases of pathologic hemorrhage thrombin is not liberated in sufficient quantity or rapidly enough to produce the desired clot." The mechanism of thrombin liberation would depend on the choice of theory of blood coagulation. Howell¹⁰ suggests that an abnormal content of antiprothrombin in hemophilia is possible which should logically require kephalin for treatment rather than a ferment (coagulose). Nevertheless, coagulose is advised by the manufacturer's literature, "in all cases of hemorrhage due to defective clotting of blood

9. Clowes and Busch: New York M. J., 1913, January 4.

10. Howell: Harvey Lectures, 1916-17, Series XII, 273-324.

as seen in hemophilia, hemorrhage of the new-born" and in fact any kind of bleeding no matter what the source, origin or cause, or to be applied locally or systemically. The clinical evidences cited by Clowes and Busch in support of the hemostatic qualities of precipitated blood serums ("coagulose") are not convincing. The alleged beneficial effects can be accounted for by other factors.

A complete record together with potency guarantees and description of the various thromboplastic preparations tested are presented in Table 1.

II. RESULTS

1. ACCELERATION OF COAGULATION TIME OF OXALATE PLASMA AND BLOOD IN VITRO BY VARIOUS THROMBOPLASTIC PREPARATIONS

The methods of testing thromboplastic activity recommended by Howell and a modification of Howell's method by Fenger described in New and Nonofficial Remedies were used with slight modifications. These may be described as follows:

Control	Agent to be Tested
Oxalated plasma.....8 drops	Oxalated plasma.....8 drops
Serum.....3 drops	Serum.....3 drops
Normal saline.....3 drops	Agent, or kephalin (0.1 %)....3 drops
Clotting with dog's and cat's blood occurs in from thirty minutes to two hours.	Clotting of same blood used in control occurs in from thirty seconds to two or three minutes.

Howell's Method.—Freshly drawn blood from cat or dog is used. Oxalated plasma and serum are secured. Blood is oxalated so as to contain about 0.1 per cent. oxalate. This is then centrifugated and the supernatant portion constitutes oxalated plasma. Blood is drawn into another cylinder and allowed to clot without interference, then centrifugalized for the serum. The control consists of water. The series for testing were made up according to McLean¹¹ as follows, using small vials about 1.2 cm. in diameter and 4.5 cm. long:

11. McLean, J.: Am. J. Physiol **10**: 250, 1916.

As control, normal saline (0.9 per cent. NaCl) was used rather than water as directed by McLean, since nearly all of the preparations tested are prepared with saline or recommended to be used in this way. In all of our work complete invertibility* was used as the end-point of coagulation, and the tests were performed at room temperature.

N. N. R. Method.—This method was used as described in New and Nonofficial Remedies.¹² To 0.5

¹² New and Nonofficial Remedies, 1919, p. 121.

c.c. of thromboplastin solution is added 24.5 c.c. normal saline and the mixture shaken until a uniform emulsion results. Of this suspension 5 c.c. is mixed in a graduated cylinder with an equal volume of blood serum. To this is added 10 c.c. of oxalated beef plasma (containing 0.1 per cent. oxalate) and mixed by inverting the cylinder twice. Instead of transferring the mixture to a shallow dish the mixture was left in the mixing cylinder and the whole immersed in a deep water bath kept at 38 C. All solutions were made fresh and saline was used for control. The test of complete invertibility was applied throughout.

In the case of preparations limited in quantity the proportions of all constituents were reduced to one fifth. The dry preparations (kephalin and coagulen) were used in the same proportions as the liquid thromboplastins, that is, 1 part of the thromboplastic preparation to 100 parts of plasma. This probably gives kephalin a higher concentration, but this could not be different owing to the unknown content of kephalin or the active constituent of thromboplastin. The comparison was made on the actual basis of method recommended for testing.

In this series of tests, the plasmas of three different cats, eight dogs and two men were used. Blood of two dogs (Experiments 18 and 20) was used as drawn from the femoral artery direct without centrifugalization. About 0.5 c.c. of the thromboplastic agent, using an equal volume of saline as control, was added to the blood directly in small vials of uniform size. The mixture was quickly shaken and complete

TABLE 1.—DESCRIPTION OF THROMBOPLASTIC AGENT AND CLAIMS FOR POTENCY

Name	Manufacturer	Record on Label	Description	Potency Guarantee	Received in Laboratory
Kephalin (fresh).....	Laboratory.....	Feb. 8, 1919.....	Light brown		Feb. 8, 1919
Kephalin (old).....	Laboratory.....	May 21, 1918.....	Deep brown		May 21, 1918
Kephalin (old).....	Armour.....	None.....	Light brown	None*.....	May 10, 1917
Kephalin (fresh).....	Armour.....	(H. W. D. Feb. 11, 1919).....	Light brown	None.....	Mar. 4, 1919
Kephalin (sheep).....	Laboratory.....	1912.....	Black, viscid, tarry.....		1912
Kephalin (pig).....	Laboratory.....	April 7, 1919.....	Nearly white, slight yellowish.....		April 7, 1919
Kephalin.....	Armour.....	"The Hemostatic Phosphatid from Spinal Cord and Brain Tissue," From Stevenson, June 6, 1918	Soft, shiny, moist brown waxy masses	None.....	April 7, 1919
Kephalin.....	Armour.....	"Hemostatic Phosphatid from Spinal Cord and Brain Tissue," April 5, 1917	Dry, slight yellowish brown waxy lumps	None.....	April 7, 1919
Kephalin.....	Armour.....	Made April 16, 1917.....	Soft, shiny, yellowish brown, moist waxy masses	None.....	April 7, 1919
Coagulen (dry, old).....	Soc. Chem. Industry, Swiss	N/4.....	Light brown and white mixture powder	None.....	Aug. 26, 1917
Coagulen (dry, fresh)...	Soc. Chem. Industry, Basle	S/6.....	White powder	None.....	Feb. 19, 1919
Coagulen (liquid)+	Soc. Chem. Industry, Basle	Op. No. 9031.....	Milky white emulsion; 20 c.c. large ampoule	None.....	Feb. 19, 1919
Coagulen (Ciba).....	Soc. Chem. Industry, Basle	12118, Nov. 12, 1917.....	1.5 c.c. of 3 per cent. solution of coagulen. Small ampoules	None.....	April 7, 1919

Coagulen tablets.....	Soc. Chem. Industry, Basle	Sample K, Dec. 14, 1917.....	Each tablet—Coagulen (Ciba, 0.5 gm. + NaCl = 0.46 gm.	None.....	April 7, 1919
Coagulen—(Kocher Fonio Ciba)	Gesellschaft für Chem. Industry, Basle	1, July, 1915.....	Light brown or yellowish moist powder; discrete brown particles	None.....	April 7, 1919
Coagulen (Ciba).....	Soc. Chem. Industry, Basle	11230, Nov. 12, 1917.....	Sterile; 20 c.c. of 3 per cent. solution of coagulen, large ampoule	None.....	April 7, 1919
Hemostatic (fresh)	Parke Davis and Company	046461-B.....	Opalescent fluid with odor of trikresol	Aug. 16, 1920	Feb. 17, 1919
Thromboplastin (fresh)	Squibb.....	12519.....	White emulsion with odor of trikresol	Aug. 8, 1920	Feb. 19, 1919
Thromboplastin (old)...	Squibb.....	11041.....	White emulsion with odor of trikresol	None.....	April 8, 1917
Thromboplastin (fresh)	Armour.....	None.....	White emulsion with odor of trikresol	Oct. 1, 1919	Feb. 17, 1919
Thromboplastin.....	Squibb, from Stevenson	10441, May 25, 1918.....	White emulsion with odor of trikresol	Dec. 19, 1918	April 7, 1919
Thromboplastin.....	Squibb, from manufacturer	7661, June 21, 1916.....	White emulsion with odor of trikresol	June 6, 1917	April 7, 1919
Thromboplastin (B)...	Armour.....	None, May 15, 1918.....	Coarse white emulsion with odor of trikresol	May 14, 1919	April 7, 1919
Thromboplastin (A)....	Armour.....	None, May 15, 1918.....	Coarse white emulsion with odor of trikresol	May 14, 1919	April 7, 1919
Coagulose.....	Parke Davis and Company	045068B.....	White powder	May 20, 1921	May 2, 1919

* None stated.

† "Coagulen-Ciba. 20 c.c. in sterile solution ready for use. To be shaken. Op. No. 9981. Importé de Suisse." This was carefully opened April 30, 1919, and contents found to measure 15 c.c. Another ampoule with the same label and Op. No. 9941 contained considerable sediment. This preparation is recommended for intravenous injection.

invertibility taken as the end-point. The saline controls were made simultaneously with the test for each agent. Experiments were also made with six different beef plasmas.

All of the data obtained are presented in Table 2. These represent averages of not less than two and in the majority of instances three trials with each agent.

The results indicate that certain of the agents, namely the tromboplastins and kephalin, though variable, definitely accelerate the coagulation time of both oxalate plasmas and blood as compared with the saline controls. The marked variations and inactivity of certain specimens of these two agents are due to deterioration on standing and will be described in Section 3.

Fresh hemostatic serum was found to shorten appreciably the coagulation time in only four out of eleven experiments, but this was much less than with kephalin and tromboplastin in the same experiments and about the same as the saline controls, indicating the weakness of the product.

Fresh dry coagulen only moderately shortened the coagulation time in three out of the nine tests made. Fresh coagulen solution (in ampules) shortened the coagulation time more than saline in only two out of seven tests that were made. Coagulen tablets were not tested since these are prepared from dry coagulen and would hardly be expected to show anything different.

Coagulose was found to be inactive with bovine and feline plasmas.

In experiments 4 and 6 observations with a miscellaneous lot of agents indicate that acceleration of coagulation time is not limited to kephalin and the thromboplastins. Among these were calcium chlorid, fresh saliva and raw and boiled milk. They possessed about one half to one third the activity of fresh kephalin under the same conditions. With milk and saliva the effects are to be attributed to calcium and the results are of interest in explaining sources of contamination and possible adulteration (milk). Such

colloidal agents as agar, starch, gelatin, acacia, excess of dog serum and also lecithin were without influence. Urine and sulphocyanid were found to prolong the time of coagulation. Apparently thromboplastic activity by kephalin and thromboplastin is not of colloidal nature and certain salts may be detrimental to the process.

CONCLUSIONS

The kephalin and thromboplastin type of thromboplastic preparations definitely and rather markedly accelerate the coagulation time of blood and oxalate plasmas *in vitro*, while coagulen, hemostatic serum and coagulose are practically inactive.

The coagulation time can also be accelerated by calcium chlorid, fresh saliva and milk, while such colloids as starch, gelatin, acacia and lecithin under the conditions appear to be inactive.

2. ACCELERATION OF COAGULATION TIME OF PEPTONE BLOOD AND PLASMA

Freshly drawn blood from dogs injected with Witte's peptone (1.5 c.c. of 20 per cent. per kilogram) was used in four experiments and the plasma in one experiment according to Howell's technic, using normal saline as control.

The results in Table 3 indicate that clotting of peptone bloods and plasma was also markedly accelerated by the thromboplastic agents. In descending order of efficiency these are thromboplastin, kephalin, and coagulen. Hemostatic serum was inactive in the single experiment performed.

3. BLOOD AND PLASMA COAGULATION EFFICIENCY OF THROMBOPLASTIC AGENTS

In this section only the freshly obtained and prepared agents are considered. The activity of old specimens is discussed in the following section. The detailed data as to the comparative efficiency of the agents (old and fresh) are presented in Table 4, and summarized and grouped in Table 5.

TABLE 2.—ACCELERATION OF COAGULATION TIME OF OXALATE PLAS

Agents and Concentrations Used	Number of Experiments							
	1	2	3†	4†	6‡	8	9	10
	Dog Plasma Howell	Dog Plasma Howell	Dog Plasma Howell	Dog Plasma Howell	Dog Plasma Howell	Beef Plasma N.N.R.	Beef Plasma N.N.R.	Beef Plasma N.N.R.
Coagulation Time in Minutes								
Kephalin (sheep, fresh, 2/8/19), 0.1%.....	3	4	3	2	7	8	12 (2%)	3½
Kephalin (pig's, fresh, 4/7/19), 0.1%.....								
Kephalin (A r m o u r, 2/11/19), 0.1%.....		6				7		5½
Kephalin (old, 5/21/18), 0.1%.....		7				7		7 (1%)
Kephalin (Armour, old, 6/6/18), 0.1%.....								
Kephalin (Armour, old, 4/5/17), 0.1%.....	20							
Kephalin (Armour, old, 4/16/17), 0.1%.....								
Kephalin (Armour, old, 5/10/17), 0.1%.....		7				25	3 (1%)	5
Kephalin (sheep, old, 1912), 0.1%.....					>35			>60
Thromboplastin (Squibb, fresh, 2/19/19), whole.....		3				½	⅔	⅔
Thromboplastin (Squibb, old, 5/25/18), whole.....								
Thromboplastin (Squibb, old, 4/8/17), whole.....	3	4				⅔	25	1½
Thromboplastin (Squibb, old, 6/21/16), whole.....								
Thromboplastin (Armour, fresh, 2/18/19), whole.....	4	4				1	2½	42
Thromboplastin (Armour, old, 5/15/18), whole.....								
Coagulen (dry, fresh, 2/19/19), 3%.....	6½ (0.1%)	8 (0.1%)	20 (5%)			22 (5%)	>204 (1%)	>60
Coagulen (dry, old, 8/26/17), 3%.....								240
Coagulen (dry, old, 7/9/15), 3%.....								
Coagulen solution (fresh ampoule, 2/19/19), whole.....		38	5			24		
Coagulen solution (old ampoule, 7/12/17), whole.....								
Coagulen solution (old ampoule, 11/12/17), whole.....								
Hemostatic serum (fresh, 2/17/19), whole	1	9	6			33		120
Coagulose (dry, fresh, 5/2/19), 6%.....								
Control (saline).....	25	26	20	12	>180	31	60	240

* The concentration of kephalin in plasma by Howell's method is estimated to be about 0.04 per cent.; in the N.N.R. method, 1 per cent.; also of the thromboplastins. The figures in parenthesis in the first column refer to number of month/day/year, i. e., age of the preparation. The name, "Howell," and the initials, "N.N.R.," in the table refer to the methods used as described in the text. The sign (>) means more than.

† The remaining results of this experiment are as follows; fresh saliva, 2½ minutes and 0.3 per cent. trikresol, ten minutes.

‡ The remaining results of this experiment are as follows (figures represent coagulation time in minutes): Fresh saliva 7½, old saliva 10, 0.1 per cent. egg lecithin 16,

MA AND BLOOD IN VITRO BY VARIOUS THROMBOPLASTIC AGENTS *

Number of Experiment									
10a¶ Beef Plasma N.N.R.	13 Cat Plasma Howell	15 Beef Plasma N.N.R.	18 Dog Blood Howell	19 Human Plasma Howell	21 Human Plasma Howell	22 Dog Blood Howell	23 Cat Plasma Howell	23a Beef Plasma N.N.R.	24 Cat Plasma Howell
Coagulation Time in Minutes									
7 $\frac{3}{8}$	1 $\frac{3}{4}$	43			7			3 $\frac{1}{2}$	15 $\frac{1}{2}$
.....	1 1/12	8	1 $\frac{1}{8}$	4	4 7/10			4	2 $\frac{1}{4}$
8	1	7	17/30 (1%)	4 $\frac{3}{4}$	8 $\frac{1}{2}$			2	
.....	2 $\frac{1}{2}$	17			8 $\frac{1}{2}$			10 $\frac{1}{2}$	
.....	57/60	6 $\frac{1}{2}$			9 $\frac{1}{4}$	11/30		2 $\frac{1}{2}$	4
.....	1	8			8 $\frac{1}{2}$	11/30		2 $\frac{1}{2}$	3 $\frac{1}{2}$
.....	1	5			9 $\frac{1}{4}$	5/12		2 $\frac{1}{4}$	3 $\frac{1}{2}$
5	1	7			8 $\frac{1}{2}$			2 $\frac{1}{2}$	
.....	4 $\frac{1}{2}$							>82	18 $\frac{1}{2}$
7/12	13/15	11/20	$\frac{1}{4}$	1 $\frac{2}{3}$	1 $\frac{1}{2}$			1 $\frac{3}{4}$	2
.....	11/12	25		4	5 $\frac{1}{2}$	$\frac{1}{2}$		10	2 $\frac{1}{2}$
2 $\frac{1}{2}$	1 $\frac{1}{4}$	2		4	4			42	
.....	1 $\frac{5}{6}$	>150		12	5 $\frac{1}{2}$	1		10	
13/15	$\frac{3}{4}$	$\frac{1}{2}$	5/12	2	1 $\frac{1}{2}$	$\frac{1}{4}$		2	1 $\frac{3}{4}$
.....	1	1		10	4 $\frac{1}{2}$			4	2 $\frac{1}{2}$
.....	4	>300	11/12	21	8 $\frac{1}{6}$			9 $\frac{1}{2}$	
.....	3 $\frac{1}{2}$	>300		12	8 $\frac{1}{6}$			11 $\frac{1}{2}$	
.....	2	>360		12	8 $\frac{1}{6}$	1 $\frac{1}{6}$		4 $\frac{1}{2}$	3 $\frac{3}{4}$
18 hrs.		>300	$\frac{3}{4}$		10				
.....		>360		12 $\frac{1}{2}$					
.....	3 $\frac{1}{2}$	>204			15				
.....	4 $\frac{1}{4}$	>360	1 1/10	12	10 7/10			14	
18 hrs.	3 $\frac{1}{2}$	>360	1	7 $\frac{1}{6}$	10	1 $\frac{2}{3}$	12 11 $\frac{1}{2}$	7 6	2 $\frac{1}{2}$

0.1 per cent. agar 20, fresh raw milk 5, fresh boiled milk 6, 5 per cent. CaCl₂ 4, 0.5 per cent. acacia 10, dog serum 25, 0.5 per cent. gelatin >12, 2 per cent. starch >22, 0.1 per cent. KSCN >43, urine >30.

§ Additional results in this experiment were: fresh raw milk 15 minutes, and fresh boiled milk 16 minutes.

¶ A 100 per cent. saline extract from fresh dog's liver coagulated this plasma in 4 minutes.

|| In experiment 10a the serum of one and the plasma of another animal were used.

Thromboplastins.—From the summary in Table 5 it is seen that the thromboplastins were distinctly superior to all other products tested, the Squibb product possessing a median efficiency of more than three times that of the Armour product and seven times that of the freshest and some old kephalins. The greater clotting accelerator activity of the thrombo-

TABLE 3.—ACCELERATION OF COAGULATION TIME OF PEP-TONE BLOOD AND PLASMA (DOG) BY VARIOUS THROMBOPLASTIC AGENTS *

Agents and Concen- trations Used	Number of Experiment				
	13	14	16	17	18
	Blood	Plasma	Blood	Blood	Blood
Coagulation Time in Minutes					
Kephalin, 0.1 per cent. (fresh, sheep, 2/8/19).....	..	>68			
Kephalin, 0.1 per cent. (fresh, pig's, 4/7/19).....	15		9	11/12	Found clotted end of 18 hrs.
Kephalin, 0.1 per cent. (fresh, Armour, 2/11/19).....	..			¾	
Thromboplastin, whole (Squibb fresh, 2/19/19).....	1	1½		1/10	
Thromboplastin, whole (Armour fresh, 2/17/19).....	..	2		¼	
Coagulen, 3 per cent. (fresh, dry, 2/19/19).....	..			1½	Not clotted end of 18 hrs.
Hemostatic serum (fresh, 2/17/19) ..	..	>60			
Control (saline).....	30	>69	16	10	Not clotted end of 18 hrs.

* Howell's method was used. The sign (>) means more than. The figures in parentheses in the first column refer to dates.

plastins, which are saline or aqueous extracts of tissue, as compared with kephalin, is confirmative of previous observations of Rumpf,¹³ who found that lipin emulsions (presumably kephalin) prepared from ox brain were far less active than tissue extracts. As compared with the saline control, the fresh thromboplastins hasten coagulation by about ten times with the Armour product and twenty times with Squibb's product.

13. Rumpf, cit. MacLean, Hugh: Loc. cit.

tion of both. Augmentation was observed in plexus free preparations with atropin, but was of less magnitude than when the plexus was intact. The toxic effect of atropin on the muscle is probably in part responsible for the decrease. The conclusion was reached that both muscle and nerve are stimulated by the bromids in plexus containing organs. Prochnow⁷ concluded that the bromids stimulate smooth muscle directly on the basis of experiments on the carotid artery of ox. Such preparations are reported to be free from intrinsic nerve cells.

DISCUSSION

In a previous communication it was shown that the bromids act as mild stimulants to skeletal muscle, cardiac and smooth muscle of the heart and to certain medullary nerve centers.⁸ In this paper it is shown that many types of smooth muscle are stimulated. The early effect of the bromids therefore appears to be a primary stimulation.

Symptoms of stimulation have been observed clinically⁹ both on the central nervous system and on the gastro-intestinal tract. Depression is a later manifestation.

The variable quantitative changes occurring in the tissue are dependent on its physiologic condition. In the strip technic the tissue goes through variable degrees of asphyxiation. A mild stimulant has accordingly a profound effect. The same principle is evident in fatigued muscle nerve preparations of a frog. It is for this reason that segments contracting arrhythmically show the most profound changes whereas the changes are less intense in rhythmic preparations.

7. Prochnow: *Arch. internat. de Pharmacol. et de Therap.* **21**: 287, 1911.

8. Kruse: *J. Pharmacol. and Exper. Therap.* **14**: 63, 1919.

9. Fere: *Nouvelle Iconographie de la Salpêtrière* **15**: 435, 1902. Mitchel: *Therap. Wehnschr.* **3**: 821, 1896. Hodges: *South Med. Record*, Atlanta, 1896, 505.

SUMMARY

1. The bromids stimulate smooth muscle slightly when the tissue is in a normal and rhythmic condition and markedly when it is arrhythmic or in a partly fatigued state.

2. The primary stimulation of the bromids seems to be a property common for many tissues.

THE PLASMA AND BLOOD CLOTTING EFFICIENCY OF THROMBOPLASTIC AGENTS IN VITRO AND THEIR STABILITY *

P. J. HANZLIK

AND

C. M. WEIDENTHAL

CLEVELAND

(From *The Journal of Pharmacology and Experimental Therapeutics*,
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I. INTRODUCTION

It was previously shown ¹ that the entire group of hemostatic agents is rather limited in their usefulness, due in part to the difficulty of applying crucial tests in determining their efficiency under actual conditions

* From the Pharmacological Laboratory, School of Medicine, Western Reserve University, Cleveland.

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* This research was supported in part by a grant from the Therapeutic Research Committee, Council of Pharmacy and Chemistry of the American Medical Association.

1. Hanzlik: *J. Pharmacol. & Exper. Therap.* **10**: 523, 1918; **12**: 71, 1918; **12**: 119, 1918.

of wound hemorrhage. This is particularly true of the thromboplastic type of agents derived from tissues or blood. Among these are kephalin, and such commercial preparations as thromboplastin, coagulen, hemostatic serum and coagulose. The claims made for the hemostatic qualities of these appear exaggerated when it is borne in mind that hemorrhages of whatever origin are most capricious, and generally tend to cease spontaneously, even in hemophilia.

There is no doubt that kephalin, at least, accelerates the clotting of oxalate and peptone plasma in the presence of fresh serum *in vitro*, and it is largely on this basis that its use in hemorrhages has been advocated, although, of course, this does not necessarily follow. Lack of success with these agents has been attributed to the use of old and deteriorated products and it is conceivable also that their activity varies with the dosage and concentration.

It is the object of this communication to compare the thromboplastic activity of several different products; the activity of fresh and old preparations, and the relation of concentration to their activity *in vitro*. Subsequent communications will deal with hemostatic properties in different kinds of hemorrhages in animals, and toxicity.

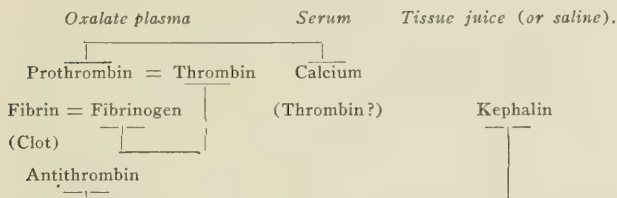
The thromboplastic agents tested were secured from various sources on the market, from the manufacturers direct, and two preparations of kephalin were made in the laboratory. These may now be described.

1. DESCRIPTION OF THE THROMBOPLASTIC AGENTS TESTED

Most of the commercial thromboplastic preparations are not secret or patented remedies, although the chemical composition of some, particularly as to active constituents, is vague or unknown. These have been evolved or inspired by reputable investigators in connection with studies on the coagulation of blood. For convenience, the various agents may be conveniently classified into three groups.

1. *Thromboplastins*.—These are saline extracts usually of brain, containing a large quantity of protein, presumably also some kephalin, possibly ferments, salts, etc. Thromboplastin was first introduced by Hess.² Two such preparations are now on the market under the name of thromboplastin.

2. *Kephalin*.—This is a lipin (mono-amino phosphatid) apparently of fairly definite chemical composition. Bang gives the formula as $C_{42}H_{80}NPO_4$, but according to Hugh MacLean considerable discrepancy exists with respect to the percentage content of carbon and hydrogen as indicated by the work of various investigators. It is prepared by ether extraction of brain (pig or sheep). It was originally described by Thudicum³ and is regarded by Howell⁴ as the con-



stituent of tissue juice responsible for the coagulation of blood. A mixture of oxalate plasma and fresh serum will clot without kephalin, but the time of clotting is greatly shortened by kephalin. It is, therefore, an accelerator of clotting. Kephalin is not appreciably present in blood, but is abundantly found in tissues, particularly the brain. According to Howell the principle rôle of kephalin is to inactivate or neutralize antithrombin allowing the prothrombin in the presence of calcium to form thrombin which in turn converts fibrinogen to fibrin, the clot. The mechanism of action may be represented schematically as follows from the actual ingredients used in Howell's test for thromboplastic activity:

2. Hess: J. A. M. A. **64**: 1395, 1915.

3. Thudicum, cited by Bang, l.: *Chemie u. Biochemie der Lipide*, 1911, Wiesbaden.

4. Howell: *Am. J. Physiol.* **13**: 1, 1912.

Other theories of blood coagulation, notably those of Morawitz and Hammarsten,⁵ are based on different conceptions. However, it is not the object of this paper to discuss the theories of blood coagulation. For these the current textbooks of physiology and works of the respective investigators may be consulted. The chemical and physical properties of kephalin are described in monographs by Bang³ and Hugh MacLean.⁶ It should be borne in mind that kephalin is insoluble in water, but swells like lecithin, forming an emulsion or colloidal suspension, which is modified by various factors. Owing to this the kephalin content of aqueous (normal saline) extracts of tissues must be variable and uncertain.

3. *Serum Products*.—To this class belong preparations known as coagulen, hemostatic serum and coagulose. These are prepared from serums of different animals, usually, from horse serum, although rabbit and human serums have also been used. The exact composition of these agents is unknown.

(a) Hemostatic serum: This is alleged to be a serum derivative discovered by Dr. Vincent A. Lapenta,⁷ composed principally of prothrombin and antiprothrombin in physiologic balanced solution. The circulars of the manufacturer make extravagant claims for this alleged hemostatic in "pulmonary hemorrhage, purpura hemorrhagica, intestinal bleeding, bleeding of the new-born, indolent ulcers, and hemorrhages incidental to various surgical procedures such as bone operations, intracranial surgery, herniotomy, tonsillectomy, amputations and hysterectomy." The experimental evidence submitted by Lapenta is uncritical and unsatisfactory.

(b) Coagulen: This preparation was inspired by Kocher and Fonio⁸ of Berne, and is alleged to be a "physiologic styptic prepared from the natural coagu-

5. Hammarsten-Hedin: *Text Book of Physiological Chemistry*, 1915, 7th ed., Wiley & Sons.

6. MacLean, Hugh: *Lecithin and Allied Substances*, 1918, Longmans, Green and Company.

7. Lapenta, V. A.: *Therapeutic Gazette* **34**: 23, 1918.

8. Fonio: *Corr.-Blatt f. Schweiz. Aerzte* **43**: 385, 422, 456, 1913.

lents of animal blood contained in the blood platelets. It has the characteristics of a lipid." If kephalin is meant, it is difficult to understand why platelets should be selected in preference to other abundantly supplied organs such as brain. The preparation is marketed in three forms: (1) as dry powder containing lactose; (2) as 3 per cent. sterile solution in ampules, and (3) tablets. The object of the lactose is stated to be for facilitating solution in water.

Coagulen is recommended for use by practically all known methods of administration and for all kinds of hemorrhages irrespective of cause, origin or etiology, with sweeping generalizations giving the impression of a panacea for bleeding ills. The same may be said of hemostatic serum and coagulose.

(c) Coagulose: This is claimed to be an acetone-ether precipitation product of normal blood serum (presumably horse serum) inspired and originated by G. H. A. Clowes and F. C. Busch⁹ and marketed under the name of "Coagulose" by Parke, Davis & Co. "It is a sterile, soluble, anhydrous powder, containing the fibrin ferment necessary for clotting blood. It is readily soluble in cold water and concentrations two or three times that of original serum concentrations . . . retaining its active principles unimpaired for long periods of time." The experimental and clinical evidences have been mainly supplied by Clowes and Busch. According to them "Practical experience would indicate that in the large majority of cases of pathologic hemorrhage thrombin is not liberated in sufficient quantity or rapidly enough to produce the desired clot." The mechanism of thrombin liberation would depend on the choice of theory of blood coagulation. Howell¹⁰ suggests that an abnormal content of antiprothrombin in hemophilia is possible which should logically require kephalin for treatment rather than a ferment (coagulose). Nevertheless, coagulose is advised by the manufacturer's literature, "in all cases of hemorrhage due to defective clotting of blood

9. Clowes and Busch: New York M. J., 1913, January 4.

10. Howell: Harvey Lectures, 1916-17, Series XII, 273-324.

as seen in hemophilia, hemorrhage of the new-born" and in fact any kind of bleeding no matter what the source, origin or cause, or to be applied locally or systemically. The clinical evidences cited by Clowes and Busch in support of the hemostatic qualities of precipitated blood serums ("coagulose") are not convincing. The alleged beneficial effects can be accounted for by other factors.

A complete record together with potency guarantees and description of the various thromboplastic preparations tested are presented in Table 1.

II. RESULTS

1. ACCELERATION OF COAGULATION TIME OF OXALATE PLASMA AND BLOOD IN VITRO BY VARIOUS THROMBOPLASTIC PREPARATIONS

The methods of testing thromboplastic activity recommended by Howell and a modification of Howell's method by Fenger described in New and Nonofficial Remedies were used with slight modifications. These may be described as follows:

Control	Agent to be Tested
Oxalated plasma.....8 drops	Oxalated plasma.....8 drops
Serum.....3 drops	Serum.....3 drops
Normal saline.....3 drops	Agent, or kephalin (0.1 %)....3 drops
Clotting with dog's and cat's blood occurs in from thirty minutes to two hours.	Clotting of same blood used in control occurs in from thirty seconds to two or three minutes.

Howell's Method.—Freshly drawn blood from cat or dog is used. Oxalated plasma and serum are secured. Blood is oxalated so as to contain about 0.1 per cent. oxalate. This is then centrifugated and the supernatant portion constitutes oxalated plasma. Blood is drawn into another cylinder and allowed to clot without interference, then centrifugalized for the serum. The control consists of water. The series for testing were made up according to McLean¹¹ as follows, using small vials about 1.2 cm. in diameter and 4.5 cm. long:

11. McLean, J.: Am. J. Physiol **10**: 250, 1916.

As control, normal saline (0.9 per cent. NaCl) was used rather than water as directed by McLean, since nearly all of the preparations tested are prepared with saline or recommended to be used in this way. In all of our work complete invertibility was used as the end-point of coagulation, and the tests were performed at room temperature.

N. N. R. Method.—This method was used as described in New and Nonofficial Remedies.¹² To 0.5

¹² New and Nonofficial Remedies, 1919, p. 121.

c.c. of thromboplastin solution is added 24.5 c.c. normal saline and the mixture shaken until a uniform emulsion results. Of this suspension 5 c.c. is mixed in a graduated cylinder with an equal volume of blood serum. To this is added 10 c.c. of oxalated beef plasma (containing 0.1 per cent. oxalate) and mixed by inverting the cylinder twice. Instead of transferring the mixture to a shallow dish the mixture was left in the mixing cylinder and the whole immersed in a deep water bath kept at 38 C. All solutions were made fresh and saline was used for control. The test of complete invertibility was applied throughout.

In the case of preparations limited in quantity the proportions of all constituents were reduced to one fifth. The dry preparations (kephalin and coagulen) were used in the same proportions as the liquid thromboplastins, that is, 1 part of the thromboplastic preparation to 100 parts of plasma. This probably gives kephalin a higher concentration, but this could not be different owing to the unknown content of kephalin or the active constituent of thromboplastin. The comparison was made on the actual basis of method recommended for testing.

In this series of tests, the plasmas of three different cats, eight dogs and two men were used. Blood of two dogs (Experiments 18 and 20) was used as drawn from the femoral artery direct without centrifugalization. About 0.5 c.c. of the thromboplastic agent, using an equal volume of saline as control, was added to the blood directly in small vials of uniform size. The mixture was quickly shaken and complete

TABLE 1.—DESCRIPTION OF THROMBOPLASTIC AGENT AND CLAIMS FOR POTENCY

Name	Manufacturer	Record on Label	Description	Potency Guarantee	Received in Laboratory
Kephalin (fresh).....	Laboratory.....	Feb. 8, 1919.....	Light brown		Feb. 8, 1919
Kephalin (old).....	Laboratory.....	May 21, 1918.....	Deep brown		May 21, 1918
Kephalin (old).....	Armour.....	None.....	Light brown	None ^s	May 10, 1917
Kephalin (fresh).....	Armour.....	(H. W. D. Feb. 11, 1919)....	Light brown	None.....	Mar. 4, 1919
Kephalin (sheep).....	Laboratory.....	1912.....	Black, viscid, tarry.....		1912
Kephalin (pig).....	Laboratory.....	April 7, 1919.....	Nearly white, slight yellowish.....		April 7, 1919
Kephalin.....	Armour.....	"The Hemostatic Phosphatid from Spinal Cord and Brain Tissue." From Stevenson, June 6, 1918	Soft, shiny, moist brown waxy masses	None.....	April 7, 1919
Kephalin.....	Armour.....	"Hemostatic Phosphatid from Spinal Cord and Brain Tissue." April 5, 1917	Dry, slight yellowish brown waxy lumps	None.....	April 7, 1919
Kephalin.....	Armour.....	Made April 16, 1917.....	Soft, shiny, yellowish brown, moist waxy masses	None.....	April 7, 1919
Coagulen (dry, old).....	Soc. Chem. Industry, Swiss	N/4.....	Light brown and white mixture powder	None.....	Aug. 26, 1917
Coagulen (dry, fresh)...	Soc. Chem. Industry, Basle	S/6.....	White powder	None.....	Feb. 19, 1919
Coagulen (liquid)†	Soc. Chem. Industry, Basle	Op. No. 9031.....	Milky white emulsion; 20 c.c. large ampoule	None.....	Feb. 19, 1919
Coagulen (Ciba).....	Soc. Chem. Industry, Basle	12118, Nov. 12, 1917.....	1.5 c.c. of 3 per cent. solution of coagulen. Small ampoules	None.....	April 7, 1919

Coagulen tablets.....	Soc. Chem. Industry, Basle	Sample K, Dec. 14, 1917.....	Each tablet—Coagulen Ciba, 0.5 gm. + NaCl = 0.46 gm.	None.....	April 7, 1919
Coagulen—(Kocher-Fonio Ciba)	Gesellschaft für Chem. Industry, Basle	1, July, 1915.....	Light brown or yellowish moist powder; discrete brown particles	None.....	April 7, 1919
Coagulen (Ciba).....	Soc. Chem. Industry, Basle	11230, Nov. 12, 1917.....	Sterile; 20 c.c. of 3 per cent. solution of coagulen, large ampoule	None.....	April 7, 1919
Hemostatic serum (fresh)	Parke Davis and Company	046461-B.....	Opalescent fluid with odor of trikresol	Aug. 16, 1920	Feb. 17, 1919
Thromboplastin (fresh)	Squibb.....	12519.....	White emulsion with odor of trikresol	Aug. 8, 1920	Feb. 19, 1919
Thromboplastin (old)...	Squibb.....	11041.....	White emulsion with odor of trikresol	None.....	April 8, 1917
Thromboplastin (fresh)	Armour.....	None.....	White emulsion with odor of trikresol	Oct. 1, 1919	Feb. 17, 1919
Thromboplastin.....	Squibb, from Stevenson	10441, May 25, 1918.....	White emulsion with odor of trikresol	Dec. 19, 1918	April 7, 1919
Thromboplastin.....	Squibb, from manufacturer	7661, June 21, 1916.....	White emulsion with odor of trikresol	June 6, 1917	April 7, 1919
Thromboplastin (B)....	Armour.....	None, May 15, 1918.....	Coarse white emulsion with odor of trikresol	May 14, 1919	April 7, 1919
Thromboplastin (A)....	Armour.....	None, May 15, 1918.....	Coarse white emulsion with odor of trikresol	May 14, 1919	April 7, 1919
Coagulose.....	Parke Davis and Company	045098B.....	White powder	May 20, 1921	May 2, 1919

* None stated.

+ "Coagulen-Ciba. 20 c.c. in sterile solution ready for use. To be shaken. Op. No. 9631. Importé de Suisse." This was carefully opened April 30, 1919, and contents found to measure 15 c.c. Another ampoule with the same label and Op. No. 9641 contained considerable sediment. This preparation is recommended for intravenous injection.

invertibility taken as the end-point. The saline controls were made simultaneously with the test for each agent. Experiments were also made with six different beef plasmas.

All of the data obtained are presented in Table 2. These represent averages of not less than two and in the majority of instances three trials with each agent.

The results indicate that certain of the agents, namely the tromboplastins and kephalin, though variable, definitely accelerate the coagulation time of both oxalate plasmas and blood as compared with the saline controls. The marked variations and inactivity of certain specimens of these two agents are due to deterioration on standing and will be described in Section 3.

Fresh hemostatic serum was found to shorten appreciably the coagulation time in only four out of eleven experiments, but this was much less than with kephalin and tromboplastin in the same experiments and about the same as the saline controls, indicating the weakness of the product.

Fresh dry coagulen only moderately shortened the coagulation time in three out of the nine tests made. Fresh coagulen solution (in ampules) shortened the coagulation time more than saline in only two out of seven tests that were made. Coagulen tablets were not tested since these are prepared from dry coagulen and would hardly be expected to show anything different.

Coagulose was found to be inactive with bovine and feline plasmas.

In experiments 4 and 6 observations with a miscellaneous lot of agents indicate that acceleration of coagulation time is not limited to kephalin and the thromboplastins. Among these were calcium chlorid, fresh saliva and raw and boiled milk. They possessed about one half to one third the activity of fresh kephalin under the same conditions. With milk and saliva the effects are to be attributed to calcium and the results are of interest in explaining sources of contamination and possible adulteration (milk). Such

colloidal agents as agar, starch, gelatin, acacia, excess of dog serum and also lecithin were without influence. Urine and sulphocyanid were found to prolong the time of coagulation. Apparently thromboplastic activity by kephalin and thromboplastin is not of colloidal nature and certain salts may be detrimental to the process.

CONCLUSIONS

The kephalin and thromboplastin type of thromboplastic preparations definitely and rather markedly accelerate the coagulation time of blood and oxalate plasmas *in vitro*, while coagulen, hemostatic serum and coagulose are practically inactive.

The coagulation time can also be accelerated by calcium chlorid, fresh saliva and milk, while such colloids as starch, gelatin, acacia and lecithin under the conditions appear to be inactive.

2. ACCELERATION OF COAGULATION TIME OF PEPTONE BLOOD AND PLASMA

Freshly drawn blood from dogs injected with Witte's peptone (1.5 c.c. of 20 per cent. per kilogram) was used in four experiments and the plasma in one experiment according to Howell's technic, using normal saline as control.

The results in Table 3 indicate that clotting of peptone bloods and plasma was also markedly accelerated by the thromboplastic agents. In descending order of efficiency these are thromboplastin, kephalin, and coagulen. Hemostatic serum was inactive in the single experiment performed.

3. BLOOD AND PLASMA COAGULATION EFFICIENCY OF THROMBOPLASTIC AGENTS

In this section only the freshly obtained and prepared agents are considered. The activity of old specimens is discussed in the following section. The detailed data as to the comparative efficiency of the agents (old and fresh) are presented in Table 4, and summarized and grouped in Table 5.

TABLE 2.—ACCELERATION OF COAGULATION TIME OF OXALATE PLAS

Agents and Concentrations Used	Number of Experiments							
	1	2	3†	4‡	6§	8	9	10
	Dog Plasma Howell	Dog Plasma Howell	Dog Plasma Howell	Dog Plasma Howell	Dog Plasma Howell	Beef Plasma N.N.R.	Beef Plasma N.N.R.	Beef Plasma N.N.R.
Coagulation Time in Minutes								
Kephalin (sheep, fresh, 2/8/19), 0.1%.....	3	4	3	2	7	8	12 (2%)	3½
Kephalin (pig's, fresh, 4/7/19), 0.1%.....								
Kephalin (Armour, old, 2/11/19), 0.1%.....		6				7		5½
Kephalin (old, 5/21/18), 0.1%.....		7				7		7 (1%)
Kephalin (Armour, old, 6/6/18), 0.1%.....								
Kephalin (Armour, old, 4/5/17), 0.1%.....	20							
Kephalin (Armour, old, 4/16/17), 0.1%.....								
Kephalin (Armour, old, 5/10/17), 0.1%.....		7				25	3 (1%)	5
Kephalin (sheep, old, 1912), 0.1%.....					>35			>60
Thromboplastin (Squibb, fresh, 2/19/19), whole.....		3				½	⅔	⅔
Thromboplastin (Squibb, old, 5/25/18), whole.....								
Thromboplastin (Squibb, old, 4/8/17), whole.....	3	4				⅔	25	1½
Thromboplastin (Squibb, old, 6/21/16), whole.....								
Thromboplastin (Armour, fresh, 2/18/19), whole.....	4	4				1	2½	42
Thromboplastin (Armour, old, 5/15/18), whole.....								
Coagulen (dry, fresh, 2/19/19), 3%.....	6½ (0.1%)	8 (0.1%)	20 (5%)			22 (5%)	>204 (1%)	>60
Coagulen (dry, old, 8/26/17), 3%.....								240
Coagulen (dry, old, 7/9/15), 3%.....								
Coagulen solution (fresh ampoule, 2/19/19), whole.....		38	5			24		
Coagulen solution (old ampoule, 7/12/17), whole.....								
Coagulen solution (old ampoule, 11/12/17), whole.....								
Hemostatic serum (fresh, 2/17/19), whole	1	9	6			33		120
Coagulose (dry, fresh, 5/2/19), 6%.....								
Control (saline).....	25	26	20	12	>180	31	60	240

* The concentration of kephalin in plasma by Howell's method is estimated to be about 0.04 per cent.; in the N.N.R. method, 1 per cent.; also of the thromboplastins. The figures in parenthesis in the first column refer to number of month/day/year, i. e., age of the preparation. The name, "Howell," and the initials, "N.N.R.," in the table refer to the methods used as described in the text. The sign (>) means more than.

† The remaining results of this experiment are as follows; fresh saliva, 2½ minutes and 0.3 per cent. trikresol, ten minutes.

‡ The remaining results of this experiment are as follows (figures represent coagulation time in minutes): Fresh saliva 7½, old saliva 10, 0.1 per cent. egg lecithin 16,

MA AND BLOOD IN VITRO BY VARIOUS THROMBOPLASTIC AGENTS *

Number of Experiment

10a¶	13	15	18	19	21	22	23	23a	24
Beef Plasma N.N.R.	Cat Plasma Howell	Beef Plasma N.N.R.	Dog Blood Howell	Human Plasma Howell	Human Plasma Howell	Dog Blood Howell	Cat Plasma Howell	Beef Plasma N.N.R.	Cat Plasma Howell

Coagulation Time in Minutes

7¾	1¾	43			7			3½	15½
.....	1 1/12	8	1½	4	4 7/10			4	2¼
8	1	7	17/30 (1%)	4¾	8½			2	
.....	2½	17			8½			10½	
.....	57/60	6½			9¼	11/30		2½	4
.....	1	8			8½	11/30		2½	3½
.....	1	5			9¼	5/12		2¼	3½
5	1	7			8½			2½	
.....	4½							>82	18½
7/12	13/15	11/20	¼	1⅔	1½			1¾	2
.....	11/12	25		4	5½	½		10	2½
2½	1¼	2		4	4			42	
.....	1⅙	>150		12	5½	1		10	
13/15	¾	½	5/12	2	1½	¼		2	1¾
.....	1	1		10	4½			4	2½
.....	4	>300	11/12	21	8⅙			9½	
.....	3½	>300		12	8⅙			11½	
.....	2	>360		12	8⅙	1⅙		4½	3¼
18 hrs.		>300	¾		10				
.....		>360		12½					
.....	3½	>204			15				
.....	4¼	>360	1 1/10	12	10 7/10			14	
18 hrs.	3½	>360	1	7⅙	10	1⅔	12 11½	7 6	2½

0.1 per cent. agar 20, fresh raw milk 5, fresh boiled milk 6, 5 per cent. CaCl₂ 4, 0.5 per cent. acacia 10, dog serum 25, 0.5 per cent. gelatin >12, 2 per cent. starch >22, 0.1 per cent. KSCN >43, urine >30.

§ Additional results in this experiment were: fresh raw milk 15 minutes, and fresh boiled milk 16 minutes.

¶ A 100 per cent. saline extract from fresh dog's liver coagulated this plasma in 4 minutes.

|| In experiment 10a the serum of one and the plasma of another animal were used.

Thromboplastins.—From the summary in Table 5 it is seen that the thromboplastins were distinctly superior to all other products tested, the Squibb product possessing a median efficiency of more than three times that of the Armour product and seven times that of the freshest and some old kephalins. The greater clotting accelerator activity of the thrombo-

TABLE 3.—ACCELERATION OF COAGULATION TIME OF PEP-TONE BLOOD AND PLASMA (DOG) BY VARIOUS THROMBOPLASTIC AGENTS *

Agents and Concen- trations Used	Number of Experiment				
	13	14	16	17	18
	Blood	Plasma	Blood	Blood	Blood
	Coagulation Time in Minutes				
Kephalin, 0.1 per cent. (fresh, sheep, 2/8/19).....	..	>68			
Kephalin, 0.1 per cent. (fresh, pig's, 4/7/19).....	15		9	11/12	Found clotted end of 18 hrs.
Kephalin, 0.1 per cent. (fresh, Armour, 2/11/19).....	..			¾	
Thromboplastin, whole (Squibb fresh, 2/19/19).....	1	1½		1/10	
Thromboplastin, whole (Armour fresh, 2/17/19).....	..	2		¼	
Coagulen, 3 per cent. (fresh, dry, 2/19/19).....	..			1½	Not clotted end of 18 hrs.
Hemostatic serum (fresh, 2/17/19) ..	..	>60			
Control (saline).....	30	>69	16	10	Not clotted end of 18 hrs.

* Howell's method was used. The sign (>) means more than. The figures in parentheses in the first column refer to dates.

plastins, which are saline or aqueous extracts of tissue, as compared with kephalin, is confirmative of previous observations of Rumpf,¹³ who found that lipin emulsions (presumably kephalin) prepared from ox brain were far less active than tissue extracts. As compared with the saline control, the fresh thromboplastins hasten coagulation by about ten times with the Armour product and twenty times with Squibb's product.

13. Rumpf, cit. MacLean, Hugh: Loc. cit.

Kephalin.—As compared with the freshest thromboplastins, the freshly prepared and obtained kephalins possessed about one-seventh to one-third the thromboplastic activity. These as well as some old specimens were found definitely to accelerate clotting when compared with the saline control. That is, the coagulation time was only about one third to one half as compared with the control.

Serum Derivatives.—The freshest specimens of dry coagulen, coagulen solution in ampules, coagulose and hemostatic serum are seen to occupy the same grouping and efficiency values (Table 5) as normal saline. In other words, these products did not accelerate the coagulation of plasmas and blood in vitro, and accordingly are regarded as entirely inert as thromboplastic agents.

CONCLUSIONS

The freshly obtained and prepared thromboplastic agents which were tested arrange themselves in descending order of plasmas and blood clotting efficiency in vitro as follows: (1) Thromboplastin (Squibb), (2) thromboplastin (Armour), (3) kephalins (fresh and some old specimens), (4) coagulen, coagulose, hemostatic serum and normal saline.

The thromboplastins possess three to seven times the accelerator clotting efficiency of kephalin and shortens the clotting time to from one twentieth to one tenth as compared with normal saline.

The kephalins (0.1 per cent., fresh and old) possess about one seventh to one third the activity of the thromboplastin products, but as compared with saline shorten the coagulation time in vitro to from one third to one half.

Freshly obtained coagulen, hemostatic serum and coagulose do not hasten coagulation time in vitro and are therefore entirely inert as thromboplastic agents. Accordingly, the potency guarantee of eighteen months for Hemostatic serum is misleading.

TABLE I—THE PLASMA AND BLOOD COAGULATION EFFICIENCY OF VARIOUS THROMBOPLASTIC AGENTS

Agents and Concentrations Used	Number of Experiment																							Median Order of Clotting of Most Efficient Value in (%)
	1	2	3	4	5	6	7	8	9	10	10a	12	13	14	15	17	18	19	21	22	23	23a	24	
Order of clotting efficiency and efficiency values (in parentheses), when fresh pig's or sheep kephalin (0.1%) = 1																								
Kephalin (sheep, fresh, 2/8/19), 0.1%	2 (1)	2 (1)	3 (1)	1 (1)	3 (1)	1 (1)	3 (1)	3 (1)	3 (1)	3 (1)	5 (1)	9 (0.02)		4 (1)	8 (1)				6 (0.67)				5 (1)	7 (0.11)
Kephalin (pig's, fresh, 4/7/19), 0.1%												6 (1)	5 (1)		5 (5.4)	3 (1)	5 (1)	3 (1)	4 (1)			6 (0.9)	2 (1)	4 (1)
Kephalin (Ar-mour, 2 11/19), 0.1%	3 (0.33)	3 (0.33)			3 (3.9)			4 (0.64)	4 (0.64)	6 (0.9)	5 (1.1)	5 (1.1)			1 (6)		3 (1.8)	4 (<1)	9 (0.55)			2 (1.8)		4 (0.9)
Kephalin (old, 5/21/18), 0.1%	1 (0.5)	1 (0.5)			3 (0.9)			5 (0.5)				12 (0.4)			6 (2.1)				9 (0.55)			12 (0.3)		6 (0.5)
Kephalin (Ar-mour, 0 1 d, 4/5 17), 0.1%												4 (1.14)			1 (5)				10 (0.5)	2 (0.5)		4 (1.4)	6 (0.36)	4 (0.9)
Kephalin (Ar-mour, 0 1 d, 4/5/17), 0.1%	5 (0.14)											5 (1.1)			5 (5.4)				9 (0.55)	2 (0.55)		4 (1.4)	5 (0.64)	5 (0.6)
Kephalin (Ar-mour, 0 1 d, 4/16/17), 0.1%												5 (1.1)			3 (8.6)				10 (0.5)	3 (0.64)		3 (1.6)	5 (0.64)	4 (0.9)
Kephalin (Ar-mour, 0 1 d, 5/10 17), 0.1%	4 (0.5)	4 (0.5)			5 (0.33)			4 (0.7)	4 (0.7)	2 (4)	4 (1.5)	5 (1.1)			5 (5.4)				9 (0.55)			4 (1.4)		4 (1)
Kephalin (sheep, old, 1912), 0.1%												12 (0.24)										>16 (<0.04)	7 (0.12)	>8 (<0.2)
Thromboplastin (Squibb, fresh, 2/19/19), whole	1 (1.3)	1 (1.3)			1 (16)			1 (12)	1 (12)	1 (5.1)	2 (7.6)	1 (1.5)	1 (15)	1 (45)	2 (43)	1 (9.1)	1 (5.3)	1 (2.4)	1 (3.1)			1 (2)	1 (1.25)	1 (7.6)
Thromboplastin (Squibb, old, 5/25/18), whole												3 (1.2)			7 (1.7)			3 (1)	5 (0.85)	4 (0.4)		12 (0.35)	3 (0.9)	4 (0.9)
Thromboplastin (Squibb, old, 4/8/17), whole	2 (1)	2 (1)			1 (13)			4 (0.5)	2 (2.3)	3 (3)	3 (3)	8 (0.86)			9 (<0.8)			3 (1)	2 (1.1)			14 (0.08)		3 (1)

4. KEEPING QUALITIES OF THROMBOPLASTIC AGENTS

These may be judged from the summary in Table 5 based on results in Table 4.

Kephalin.—It is seen that the fresh sheep and pig kephalins, made in the laboratory, as compared with

TABLE 5.—SUMMARY OF THE COAGULATION EFFICIENCY OF VARIOUS THROMBOPLASTIC AGENTS

Group	Descending Order of Efficiency (1 = Most Efficient)	Agent	Brand	Date Obtained or Pre- pared†	Approxi- mate Age, Months
1. (Strongest)	1 (7.6)*	Thrombo- plastin	Squibb, fresh	2/19/19	Fresh
2. (Stronger)	2 (3)	Thrombo- plastin	Armour, fresh	2/19/19	Fresh
	3 (1)	Kephalin	Sheep, fresh..	2/ 8/19	Fresh
	3 (1)	Thrompo- plastin	Squibb, old...	4/18/17	22
	4 (1)	Kephalin	Pig's, fresh...	4/ 7/19	Freshest
	4 (1)	Kephalin	Armour, old..	5/10/17	21
3. (Strong)...	4 (1)	Thrombo- plastin	Armour, old..	5/15/18	9
	4 (0.9)	Kephalin	Armour, fresh	2/11/19	Fresh
	4 (0.9)	Kephalin	Armour, old..	6/ 6/18	8
	4 (0.9)	Kephalin	Armour, old..	4/16/17	22
	4 (0.9)	Thrombo- plastin	Squibb, old...	5/25/18	9
4. (Weak)....	5 (0.6)	Kephalin	Armour, old..	4/ 5/17	22
	6 (0.5)	Kephalin	Sheep, old....	6/ 6/18	8
	6 (0.4)	Coagulen	Ciba; dry.....	2/19/19	Fresh
5. (Practi- cally inac- tive)	6 (0.4)	Coagulen solution	C i b a; am- poule	2/19/19	Fresh
	6 (0.4)	Hemostatic serum	Parge, Davis & Company	2/17/19	Fresh
	6 (0.36)	Control sa- line; also Coagulose	Parke, Davis & Company	5/ 2/19	Fresh
	8 (0.3)	Thrombo- plastin	Squibb, old...	6/21/16	32
	8 (0.3)	Coagulen	Ciba; dry, old	7/ 9/15	43
	>8 (<0.2)	Kephalin	Old, sheep's...	1912	7 years
6. (Entirely inactive)	10 (0.3)	Coagulen	Ciba; dry, old	8/26/17	18
	11 (<0.3)	Coagulen solution	Ciba; old am- poule	4/12/17	22
	12 (<0.25)	Coagulen solution	Ciba; old am- poule	11/12/17	22

* Numbers in parentheses constitute the median efficiency values. The sign (>) means greater than; the sign (<) less than.

† The figures read number of month/day/year.

the fresh thromboplastins, possessed about one-seventh of the thromboplastic activity of the Squibb specimen and one-third of that of Armour's and about as strong as thromboplastins nine to twenty-two months old.

Three preparations of kephalin (Armour) which had stood for eight, twenty-one and twenty-two months, respectively, were practically as active as two specimens made freshly in the laboratory and also a fresh specimen of Armour's. Two other specimens, one of which was made in the laboratory, and the other obtained from Armour & Co. and which were 8 and 22 months old, respectively, at the time of testing, possessed only about one half the activity of the freshest kephalins. The oldest kephalin prepared in the laboratory seven years ago was entirely inactive. It is indicated by the results in Table 2 that the fresh kephalin prepared from sheep's brain early in February had lost considerable activity by the end of May, i. e., in about four months, although with some plasmas and blood its original activity was nearly restored during April and May. Fresh kephalin from pig's brain, which was prepared when it appeared that the sheep kephalin was losing strength, was certainly stronger than the latter, but after standing for about two months it also lost appreciably in activity.

It appears, therefore, that some specimens of kephalin lose their thromboplastic activity gradually on standing, this being demonstrable at the end of about two months. Other specimens that have stood for eight to twenty-two months are somewhat less active than freshly made specimens, but the difference is practically negligible. Still other specimens 8 to 22 months old are very much (about one half) weaker. Very old specimens, such as one of ours that had stood seven years, are very apt to be inert. Deterioration of kephalin is, therefore, a factor that needs to be seriously considered in marketing this product. These results on deterioration of kephalin obtained by us are fairly confirmative of those obtained by Howell and co-workers. What limitation should be placed on the period of activity of kephalin is difficult to state at this time owing to the scarcity of data. This may not be feasible also until more is known about kephalin and the methods of purification. However, it would appear from our

results that kephalin which has stood a year may have lost considerable activity and should be discarded. Finally, the product should be tested according to some well-known method (Howell's or N. N. R.).

Thromboplastins.—The fresh specimens were found to be the most active of all agents tested. Squibb's preparation was over seven times and Armour's about three times as active as kephalin (Table 5). Specimens from both manufacturers which were 9 and 22 months old possessed only about one eighth to one third the activity of the fresh specimens, but about the same activity as kephalins freshly made in the laboratory. A specimen of thromboplastin (Squibb) that was 32 months old was entirely inactive. From this it appears that the thromboplastins lose their plasma clotting activity on standing, and to a greater extent than a number of the kephalins studied. As indicated in Table 4, six specimens of kephalin, whether freshly prepared or 9 to 22 months old, were about equally active. However, the kephalins seemed to exhibit greater variability than the thromboplastins. Considering the variability of the individual results, and the fact that with some bloods a very old (22 months) specimen of thromboplastin was about as active as the freshest specimens of both the thromboplastin and kephalin it appears that the potency guarantees of nine months (Armour) and of one year (Squibb) furnished by the manufacturers of thromboplastins is about correct, although specimens 9 months old were less active.

Serum Products.—From the grouping in Table 5 it is seen that different specimens of coagulen, dry, solution in ampules, both freshly obtained and old; fresh hemostatic serum and coagulose were practically, if not entirely, inactive. These were no more active than the normal saline used as control. The effect of various concentrations (high and low) concerning which data are presented in Table 6 did not alter the efficiency position of these agents. In practically every experiment out of the entire series of twenty-

one performed, coagulen, and hemostatic serum always occupied the same position as saline. Coagulose was tried out in only two experiments, but the number of tests performed is sufficient to indicate the worthlessness of this product. It is to be noted that the ten different fresh specimens of hemostatic serum used possessed potency guarantees until Aug. 16, 1920, that is, about eighteen months after delivery by the manufacturer. Experiments with different concentrations to be presently described indicate the total worthlessness of coagulen, hemostatic serum and coagulose, fresh or old, as clotting accelerators of blood and plasma.

CONCLUSIONS

Both the kephalins and thromboplastins lose their activity on standing. This appears to be more variable with kephalin, since some specimens 9 to 22 months old were as active as the freshest, while other specimens of same age were much less (about one half) active. Deterioration of kephalin is demonstrable at the end of about two months on standing.

Specimens of thromboplastin from different sources 9 to 22 months old possessed about one eighth to one third the activity of the freshest specimens from the same sources and the same activity as the freshest kephalin.

An old (32 months) thromboplastin and a very old kephalin (seven years) were found to be entirely inactive.

Fresh or old coagulen (dry and solution in ampules), fresh hemostatic serum and fresh coagulose were found to possess no demonstrable thromboplastic activity in vitro.

5. RELATION OF CONCENTRATION OF THROMBOPLASTIC AGENTS TO THE COAGULATION TIME OF DIFFERENT PLASMAS

It is conceivable that the results with the less active thromboplastic agents might be different if higher concentrations are used. It will be recalled that only a 0.1 per cent. concentration of kephalin was used in

of kephalin in water, would more nearly approach the weaker than higher concentrations of kephalin emulsions. The results obtained do not sustain this hypothesis.

The matter was tested by observing the coagulation time of different plasmas, using both Howell's and the N. N. R. method, and different concentrations of kephalin ranging from 0.002 per cent. to 10 per cent. The concentrations used represent end concentrations in plasma. The results of all the experiments with the different agents tested are presented in Table 6, and in the form of curves in the accompanying figure for Experiments 10 and 11 with the thromboplastins and kephalins.

These indicate rather strikingly that the behavior of the kephalins is different from the thromboplastins. With kephalin there appears to be an optimal range of concentration. That is, the greatest acceleration of coagulation is obtained with a range of concentrations from 0.06 per cent. to 1 per cent. On either side of these limits, that is, with concentrations lower than 0.06 per cent. (down to 0.002 per cent.) and higher than 1 per cent. (up to 10 per cent., inclusive) the coagulation time is gradually and definitely prolonged. The phenomenon was rather uniform with four different specimens of kephalin, both old and fresh.

On the other hand, the behavior of the thromboplastins was entirely different. While the acceleration of coagulation by the kephalins was in a sense inversely proportional to the concentration of the phosphatid, the acceleration of coagulation by the thromboplastins was directly proportional to the concentration. The relation is not strictly mathematical, but, in general, it can be said that the higher the concentration the greater the acceleration of coagulation, i. e., a shorter coagulation time with the thromboplastins. Concentrations of the thromboplastins lower than 0.25 per cent. were not tried, since lower concentrations gave progressively a longer coagulation time. Therefore, it does not appear incorrect in principle to use relatively higher concentrations of thromboplastin extracts in

TABLE 6.—THE RELATION OF CONCENTRATION OF THROMBOPLASTIC AGENTS TO THE COAGULATION TIME OF DIFFERENT PLASMAS

[illegible]

* The sign ($>$) means more than.

plasma for comparison with weaker percentage concentrations of kephalin. In other words, low concentrations (0.1 per cent. and even less) of kephalin are entirely adequate for illustrating the maximal acceleration of coagulation power of this agent. This would not necessarily be the case if the kephalin content or the composition of the thromboplastin type of preparations was known. The curves in the accompanying figure illustrating the action of the thromboplastins indicate a different mechanism of action from that of kephalin. This would be contrary to the conception of Howell, according to whom the thromboplastic activity of tissue juice, as represented by the thromboplastic preparations, is due to kephalin, which appears to be a fairly definite chemical entity. Referring to kephalin, Hugh MacLean states "it seems probable that lipins do play some part in the coagulation of the blood, but it does not seem certain that it is the lipins which constitute the thromboplastic substance of the tissues." It is conceivable that the presence of other constituents might alter the behavior of kephalin in tissue juice (thromboplastin).

The results with coagulen, coagulose and hemostatic serum, though limited, indicate no uniform activity with either high or low concentrations. They are probably inert in any concentration.

CONCLUSIONS

The lag in thromboplastic activity exhibited by kephalin as compared with the thromboplastins is not due to the use of low concentrations.

The range of optimal concentrations of kephalin for hastening the coagulation time of plasma is about 0.06 per cent. to 1 per cent. Outside of these limits the coagulation is retarded.

The coagulation accelerator activity of the thromboplastins is directly proportional to the concentrations, indicating a difference in mechanism of action from kephalin.

Coagulen, coagulose and hemostatic serum, in both high and low concentrations gave variable results, indi-

cating on the whole the total lack of accelerator thromboplastic activity in vitro of these agents.

III. SUMMARY

1. The kephalin and thromboplastin type of thromboplastic agents definitely and rather markedly accelerate the coagulation time of blood and oxalate plasma in vitro, while coagulen, hemostatic serum and coagulose are practically inactive.

2. In descending order of thromboplastic activity with peptone plasma the agents tested arrange themselves as follows: thromboplastins, kephalin, coagulen and hemostatic serum (inactive).

3. The freshly obtained or prepared thromboplastic agents tested arrange themselves in descending order of plasma and blood clotting efficiency in vitro as follows: (1) thromboplastin (Squibb); (2) thromboplastin (Armour); (3) kephalins (fresh and some old specimens, Armour, etc.); (4) coagulen-(Ciba); (5) coagulose, and hemostatic serum (Parke Davis & Co.) and normal saline. The thromboplastins possess three to seven times the accelerator clotting efficiency of kephalin and shorten the coagulation of from one twentieth to one tenth as compared with normal saline. The kephalins (0.1 per cent. fresh and some old) possess about one seventh to one third the activity of the thromboplastins, but as compared with saline shorten the coagulation time to from one third to one half. Fresh coagulose, hemostatic serum and coagulen did not accelerate clotting. Therefore, these are inert as thromboplastic agents.

4. Both the kephalins and thromboplastins lose their thromboplastic activity on standing. This appears to be more variable with kephalin, since some specimens 9 to 22 months old were as active as the freshest, while other specimens of same age were much less (about one half) active. Deterioration of kephalin is demonstrable at the end of about two months on standing. Specimens of thromboplastin from different sources 9 and 22 months old possessed about one eighth to one third the activity of the freshest speci-

mens from the same sources and the same activity as the freshest kephalin. An old thromboplastin (32 months) and a very old kephalin (7 years) were entirely inactive. Fresh or old coagulen (dry or solution in ampules), fresh hemostatic serum and fresh coagulose possessed no demonstrable thromboplastic activity in vitro.

5. The range of optimal concentrations of kephalin for hastening the coagulation time of plasma is about 0.06 per cent. to 1 per cent. Outside of these limits the coagulation is retarded. The coagulation accelerator activity of the thromboplastins (Armour and Squibb) is directly proportional to the concentration indicating a difference in mechanism of action from kephalin. Coagulen, coagulose and hemostatic serum in both high and low concentrations gave variable results, indicating on the whole a total lack of accelerator thromboplastic activity in these agents.

HEMOSTATIC PROPERTIES OF THROMBOPLASTIC AGENTS UNDER DIFFERENT CONDITIONS *

P. J. HANZLIK

AND

C. W. WEIDENTHAL

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I. INTRODUCTION

Practically all of the thromboplastic agents, though differing in origin and composition, are claimed to be specific for or indicated in the treatment of hemophilia, melena neonatorum, etc. In fact, these remedies are advised for all hemorrhagic tendencies, and also in hemorrhages of normal individuals in whom the tendency to hemostasis has been abundantly met by the natural thromboplastic activity of tissue juice. Under these conditions it appears that the use of thromboplastic agents is irrational and not indicated. This has been pointed out by Professor Howell in his Harvey Lecture of 1917. On the other hand, if it can be shown that a hemorrhagic tendency actually represents deficiency of kephalin, or if the mechanism through which kephalin and similar agents act is unfavorably balanced, such, for instance, as an excess

* From the Pharmacological Laboratory, School of Medicine, Western Reserve University, Cleveland.

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of antiprothrombin which, according to Howell,¹ may be the case in hemophilia, then kephalin and similar agents would be logically indicated. However, the proof that these disturbances exist in or constitute the etiology of hemophilia does not as yet appear to be sufficiently well established. The scarcity of hemophilia material limits the testing of the thromboplastic agents as hemostatics to ordinary (normal) bleeding. This serves, at least, to test the favorable claims made for these agents in bleeding from surgical wounds of normal individuals.

The ineffectiveness of kephalin and thromboplastin (Squibb) in this direction was indicated in previous studies² on quantitative changes in bleeding from superficial wounds of the dog's pad. Owing to the irrigation of most of the pads in these experiments with 1 per cent. citrate, which in this concentration has been found³ to interfere with the activity of kephalin, a final decision concerning its hemostatic qualities could not be made. It was decided, therefore, to extend the experiments, using the agents in saline instead of citrate and under conditions which would be as favorable as possible and yet preserve the quantitative features. To a certain extent the original difficulties have been overcome though not ideally. The wounds under these conditions do not represent bleeding wounds in clinical practice. However, with irrigated wounds it is at least possible to obtain controls of the bleeding from one and the same wound. The bleeding from different wounds even though made to appear alike as possible is variable so that without some kind of controls the results are meaningless. Besides irrigation experiments, observations were also made along clinical lines on hemorrhages from large arteries, liver and bone wounds and on intestinal bleeding in a case of hemophilia. However, the results

1. Howell: Harvey Lectures, 1916-1917, Series XII, pp. 273-324.

2. Hanzlik, J. Pharm. Exper. Therap. **12**: 71, 1918, and **12**: 119, 1918.

3. Waksman: Am. J. Physiol. **46**: 375, 1918.

obtained under all of these conditions were not gratifying. These and the different methods used may now be described. For a description of the agents used and the sources from which these were obtained the preceding paper ⁴ of this series may be consulted.

II. RESULTS

1. EFFECT OF THROMBOPLASTIC AGENTS ON SUPERFICIAL HEMORRHAGE FROM THE DOG'S FOOT-PAD

The favorable results in the preceding paper ⁴ of this series with kephalin and the thromboplastins on oxalate plasmas and peptone bloods in vitro indicate that these agents might act as hemostatics when applied to wounds irrigated under favorable conditions. That is, in the act of continuous irrigation a constant and sufficient concentration of the thromboplastic agents around the bleeding vessels is assured, while a single application is apt to be washed away or diluted and bleeding will continue, assuming, of course, that the surrounding tissue juice is weak in thromboplastic activity.

The technic of the experiments on dogs was the same as that previously described, except that the irrigations were made with the agents in saline, using normal saline as control. Instead of quantitative estimations or urea-nitrogen or hemoglobin of the wash-fluid, the time of cessation of bleeding (bleeding time) from the denervated foot-pad was used as an index of hemostasis. Wounds of normal animals irrigated with saline cause too much clot formation in the pad wash-fluid rendering colorimetric estimation of hemoglobin unsatisfactory, and the urea content of the agents themselves makes the use of bleeding time preferable. Care was taken to exclude any effect that might be due to changes in systemic blood pressure, which was recorded in the usual way from the carotid artery. The results of the four experiments performed are presented in Table 1.

4. Hanzlik and Weidenthal: *J. Pharm. Exper. Therap.*, **14**: 81, 1919.

TABLE 1.—EFFECT OF THROMBOPLASTIC AGENTS ON BLEEDING TIME FROM THE EXPOSED DOG'S FOOT PAD

Agent and Concentration Used	Average Bleeding Time, Minutes	Hemostatic Efficiency Value (when Kephalin = 1)	Descending Order of Hemostatic Efficiency
Experiment 16 (dog, 8.2 kg.) (rate of irrigation 8 to 10 c.c. per minute)			
Saline.....	16 (2) [*]	0.44	1. Kephalin
Kephalin, 0.1 per cent. (fresh, pig's)	7¼(2)	1.0	2. Saline
Experiment 17 (dog, 6.2 kg.) (rate of irrigation 8 to 10 c.c. per minute)			
Saline.....	3 (4)	0.83	1. Kephalin
Kephalin, 0.1 per cent. (fresh, pig's)	2½(2)	1.0	Saline
Experiment 18 (dog, 10 kg.) (irrigation slow, 14 drops per minute)			
Saline.....	16 (4)	0.63	1. Thromboplastin (Squibb)
Coagulen, 3 per cent. (fresh, 2/19/19)†	>17 (1)	0.6	
Thromboplastin (Squibb, fresh, whole, 2/19/19)	4 (2)	2.5	2. Thromboplastin (Armour)
Thromboplastin (Armour, whole, fresh, 2/17/19)	1 (1)	1.4	3. Kephalin (fresh), pig's
Coagulen solution, 3 per cent. (fresh, 2/19/19)	17½(1)	0.57	4. Hemostatic serum
Kephalin, 1 per cent. (Armour, fresh, 2/11/19)	15½(1)	0.64	Kephalin (Armour, fresh) Saline
Hemostatic serum (fresh, 2/11/19)	>15 (1)	<0.66	5. Coagulen (dry, fresh) Coagulen solution (ampoule)
Kephalin, 1 per cent. (fresh, pig's)	10 (1)	1.0	
Experiment 22 (dog, 12.5 kg.) (slow irrigation, 16 drops per minute)			
Saline.....	5½(2)		1. Kephalin (Armour, 4/16/17) Thromboplastin (Armour, 5/1/18)
Kephalin, 0.1 per cent. (Armour, old, 6/6/18)	6 (2)		
Kephalin, 0.1 per cent. (Armour, old, 4/5/17)	5½(2)		2. Kephalin (Armour, 4/5/17) Saline
Kephalin, 0.1 per cent. (Armour, old, 2/16/17)	5 (2)		3. Kephalin (Armour, 6/21/18)
Coagulen, 0.1 per cent. (dry, old, 7/9/15)	7 (2)		4. Coagulen 0.1 per cent. (7/9/18) Thromboplastin (Squibb, 5/25/15)
Coagulen (same) 3 per cent.	8 (1)		
Thromboplastin (Armour, old, 5/1/18)	5 (2)		5. Coagulen 3 per cent. (7/9/15)
Thromboplastin (Squibb, old, 6/21/16)	9 (1)		6. Thromboplastin (Squibb, 6/21/16)
Thromboplastin (Squibb, old, 5/25/18)	>7 (1)		

* Figures in parentheses refer to number of observations. The sign (>) means more than; the sign (<) less than.

† These figures in all the tables represent dates on containers; "2/19/19" is to be read Feb. 19, 1919.

As was anticipated, owing to the variability in bleeding when the pads are irrigated with saline the results are not as satisfactory as when irrigated with citrate. At best a moderate degree of hemostatic activity of the agents tested is indicated. Bleeding stopped promptly when the pads were irrigated with saline alone. In Experiment 18, the thromboplastin seemed to possess considerable advantage over saline. In the remaining experiments the results with the different agents are not as striking and might well be within experimental error no matter whether the irrigations were made slowly, allowing greater opportunity for clot formation and attachment, or rapidly. The rate of irrigation is not essential, since blood clots formed on wounds even with rapid saline irrigations.

All of the agents used in each experiment have been arranged in the order of the descending efficiency, allowing in this way every possible advantage as indicated by the numerical results. In this way, while considerable variability is still apparent, yet, on the whole, the agents arrange themselves in about the same way as the results *in vitro* indicated in the previous paper.⁴ That is, the thromboplastins and kephalin are the most active, followed by saline, and finally by coagulen and hemostatic serum as inactive.

In order that small differences in hemostasis which might not be sufficiently ascertained by this method might be detected, the experiments were altered so as to permit of quantitative observations over longer periods. This was done by rendering the blood non-coagulable with injections of peptone in the animals and collecting the irrigation fluids from the pads for definite periods of time and estimation of hemoglobin in these as best as possible colorimetrically. In the method previously described urea-nitrogen was estimated in the irrigation fluids, but this did not seem feasible with tissue extracts and the serum products. Hemoglobin estimations were made by Palmer's method and were hampered by cloudiness from kephalin principally. The remaining agents did not seriously interfere. The value of 100 per cent. was

assigned to the first control irrigation fluid and the remaining irrigations were compared with this, representing, therefore, relative percentage changes in bleeding.

The results obtained and presented in Table 2 are not ideal, but indicate in a general way the tendency

TABLE 2.—EFFECT OF THROMBOPLASTIC AGENTS ON SUPERFICIAL HEMORRHAGE FROM THE FOOT PADS OF PEPTONIZED DOGS

Agent and Concentration Used	Duration of Irrigation Minutes	Change in Hemorrhage Efficiency		Descending Order of Hemostatic Efficiency
		(+ = Increase; — = Decrease) per Cent.	Value (when Kephalin = 1)	
Experiment 13 (dog, 7.2 kg.)				
Saline.....	10	—21	About 2.0	
Saline.....	20	—53		
Kephalin, 0.1 per cent. (fresh, sheep)	12½	—11	About 0.1	1. Saline
Saline.....	17	—22.5	About 1.12	2. Kephalin
Saline.....	28½	—30		
Kephalin, 0.1 per cent. (fresh, sheep)	15½	—20	About 1.0	
Experiment 16 (dog, 8.2 kg., peptonized) (Irrigation rate = 8 to 10 c.c. per minute)				
Saline.....	10	—30	About 1.0	
Saline.....	44½	—30	<0.5*	1. Kephalin
Kephalin, 0.1 per cent. (fresh, pig's)	20	—58	1.0	2. Saline
Experiment 17 (dog, 6.2 kg.) (Irrigation rate = 8 to 10 c.c. per minute)				
Saline.....	36	+142	About 0.3	
Kephalin, 0.1 per cent. (fresh pig's)	40	—45	1.0	1. Thromboplastin (Armour) Coagulen
Saline.....	6½	—28	>0.6	
Saline.....	11½	No change	0	2. Kephalin
Thromboplastin (Armour, fresh, 2/17/19)	17	{First +62 }Later —100	2.2	3. Saline
Saline.....	5	—64	>1.4	
Coagulen, 3 per cent. (fresh, dry, 2/19/19)	10½	—100	>2.2	

* The various signs have meanings as follows: (>) = more than; (<) = less than.

exhibited by the agents in unpeptonized animals and in vitro. In two out of three experiments kephalin was found to possess a greater hemostatic efficiency than in the saline controls. In the most satisfactory experiment performed (Experiment 17) thromboplastin (Armour) was superior to all the agents tried

and coagulen seemed to behave about like thromboplastin. The tendency exhibited by the results of this experiment confirms the results of Experiments 18, 16, 17 and to some extent Experiment 22 in which unpeptonized animals and saline irrigations were used.

CONCLUSIONS

The hemostatic effect of the thromboplastic agents tested on superficial hemorrhage from the dog's pad, although limited and variable in general tend to agree with their power to accelerate coagulation of blood and oxalate plasma *in vitro*.

Accordingly, the thromboplastins and kephalin are among the most active, followed by saline and coagulen and hemostatic serum as either inactive or doubtful. However, these results do not necessarily furnish the indication or rationale for usage in clinical practice.

2. EFFECT OF VARIOUS THROMBOPLASTIC AGENTS ON HEMORRHAGE FROM FEMORAL ARTERIES

To meet the objection that irrigation wounds do not meet the requirements of clinical conditions the use of a method practiced by Hirschfelder⁵ was resorted to. By this method Hirschfelder reported favorable hemostatic effects from kephalin in arterial bleeding. The idea appears to be that the dissected and exposed Scarpa's triangle offers a favorable site for clot formation and attachment which would not be the case with wounds exhibiting a free flow of blood, or with irrigations.

This method consists in exposing the femoral arteries in the Scarpa's triangles of both extremities of the dog by dissection, leaving an excavated recess in which the blood will connect and the clot attach itself to the walls. One artery is painted with kephalin or the thromboplastic agent and the other is left untreated. In our experiments this artery was always flooded with saline as control. The arteries are then transected and the coagulation time and bleeding time observed, taking note of the blood lost by siphoning into graduated vessels. According to Hirschfelder the blood

5. Hirschfelder: *Lancet* 2: 542, 1915; *Berl. klin. Wchnschr.* 52: 976, 1915.

from the artery painted with kephalin exhibits a shorter coagulation time and bleeding time and the quantity of blood lost is less than from the untreated artery.

All told, sixteen experiments were made on dogs and cats.

Thirteen experiments were made with kephalin, the thromboplastins (Armour and Squibb), coagulen and hemostatic serum, and three without the use of any agents whatever.

The thromboplastic agents were dissolved or suspended in saline and applied to one artery and an equal quantity of saline to the other artery. The escaping blood was collected in tared shallow pans placed under the gluteal regions, using a partition of pasteboard in the median line in order to prevent mixing of blood from the two wounds. The initial blood pressure was recorded from the carotid artery of each animal, and previously lowered in some, since it was believed that low levels of pressure would offer more favorable conditions for clotting, therefore, hemostasis. In other respects the technic was carried out in the same way as described by Hirschfelder.

As would be expected, it was impossible to obtain wounds which were exactly alike. Furthermore, shreds of tissue are apt to predominate in one wound more than in the other and one artery is apt to retract more than the other thereby facilitating hemostasis. If the severed end of the blood vessel happens to be covered by a pledget or fold of tissue this also tends to stop bleeding. These factors, it is believed, will account for the great variability in results obtained with treated and untreated vessels. As a rule, the animals did not struggle, but in dogs there was considerable spurting of blood when the blood pressure was high, rendering accurate collections of blood impossible. Despite these defects the method was used and the agents tested owing to the claims that have been made for kephalin. The results are presented in Table 3.

Kephalin.—In two animals with low blood pressure and treated with concentrations of 2 per cent. and 50

TABLE 3.—EFFECT OF VARIOUS THROMBOPLASTIC AGENTS
ON HEMORRHAGE FROM FEMORAL ARTERIES IN
SCARPA'S TRIANGLES

Agent and Control Used		Femoral Artery Which Was Made	Coagulation Time, Minutes	Bleeding Time, Minutes	Quantity of Blood Lost, Gm.	Level of Systemic Blood Pressure at Time of Application of Agents, Mm. Hg
Experiment	Quantity and Concentration Used					
Experiment 16 (dog, 8.2 kg., peptonized)						
Kephalin (fresh, pig's).....	5.0 (2%)	Left	3	3		96
Normal saline*.....	5.0	Right	3	3		
Experiment 17 (dog, 6.2 kg., peptonized)						
Kephalin (fresh, pig's).....	5.0 (50%)	Right	11	11	65.0	60
Experiment 16 (dog, 8.2 kg., peptonized)						
Normal saline.....	5.0	Left	7	7	66.0	
Experiment 33 (dog, 10 kg.)						
Kephalin (Armour fresh, 2/11/19).....	5.0 (50%)	Left	5	>5	237.0	110
Normal saline.....	5.0	Right	2	5	41.0	
Experiment 20 (dog, 18 kg.)						
Thromboplastin (Armour, fresh, 2/17/19).....	0.5 (whole)	Left	3	3	75.0	15
Normal saline.....	0.5	Right	3	3	50.0	
Experiment 22 (dog, 12.5 kg.)						
Thromboplastin (Squibb, fresh, 2/17/19).....	0.5 (whole)	Left	1/4	1/2	11.5	104
Normal saline.....	0.5	Right	4 1/2	6	267.2	
Experiment 26 (cat, 616 gm.)						
Thromboplastin (Squibb, fresh, 2/19/19).....	0.5 (whole)	Left	3 1/4	5 3/4	12.1	140
Normal saline.....	0.5	Right	3 1/4	5 3/4	3.0	
Experiment 32 (dog, 7 kg.)						
Thromboplastin (Armour, fresh, 2/17/19).....	1.0 (whole)	Left	2 1/2	5	67.0	120
Normal saline.....	1.0	Right	5	5	411.0	
Experiment 35 (dog, 15 kg.)						
Thromboplastin (Armour, fresh, 2/17/19).....	5.0 (whole)	Left	1/2 to 2	2	47.0	112
Normal saline.....	5.0	Right	3	3	681.0	
Experiment 36 (dog, 13 kg.)						
Thromboplastin (Armour, fresh, 2/17/19).....	5.0 (whole)	Right	2	75	831.0	120
Normal saline.....	5.0	Left	1	2	387.0	
Experiment 27 (cat, 635 gm.)						
Coagulen (fresh, dry, 2/19/19).....	0.5 (3%)	Left	2	2 1/2	5.0	110
Normal saline.....	0.5	Right	2	2 1/2	12.6	
Experiment 34 (dog, 12 kg.)						
Coagulen (fresh, dry, 2/19/19).....	5.0 (5%)	Left	1	2 1/2	267.0	100
Normal saline.....	5.0	Right	1	2 1/2	273.0	
Experiment 28 (cat, 740 gm.)						
Hemostatic serum (fresh, 2/17/19).....	0.5 (whole)	Left	2 3/4	5 1/4	17.8	110
Normal saline.....	0.5	Right	2 3/4	5 1/4	13.3	
Experiment 37 (dog, 5 kg.)						
Hemostatic serum (fresh, 2/17/19).....	2.5 (whole)	Right	3	3	231.0	120
Normal saline.....	2.5	Left	1	1	43.0	
Experiment 29 (dog, 7 kg.)						
None.....	None	Left	17	17	117.0	80
None.....	None	Right	2 1/2	3	150.0	
Experiment 30 (dog, 10 kg.)						
None.....	None	Left	3 1/2	1	247.0	163
None.....	None	Right	3 1/2	1	50.0	
Experiment 31 (dog, 12 kg.)						
None.....	None	Left	5	2	337.0	120
None.....	None	Right	5	2	221.0	

* 0.9 per cent. NaCl. The sign (>) means more than.

per cent. of fresh kephalin the coagulation time and bleeding time, as far as could be judged, were about the same from both vessels. The coagulation time of the blood of these animals, which were peptonized, was markedly accelerated by kephalin in vitro (see previous paper ⁴). In another animal the bleeding from the artery treated with saline stopped sooner and the loss of blood was about one sixth of that from the kephalinated vessel.

Thromboplastin.—Five dogs and one cat with blood pressures ranging from 15 to 140 mm. were treated with fresh thromboplastins (Armour and Squibb), using different quantities. The coagulation time and bleeding time in three animals (Experiments 20, 26 and 35) were practically unchanged. In another animal (Experiment 36) the coagulation time and bleeding time were markedly shortened by saline. In only one (Experiment 22) out of six animals were the coagulation time and bleeding time from the vessel treated with thromboplastin markedly shortened and the loss of blood was much less than from the control. In two other animals (Experiments 32 and 35) the coagulation time was shortened somewhat, the bleeding time being unaltered and the loss of blood was less than from the control arteries. Therefore, it would appear that 50 per cent. of the animals whose arteries were treated with thromboplastins yielded results more or less favorable to the hemostatic action of these agents.

However, this is doubtful when bleeding from animals whose vessels were not treated at all (Experiments 29, 30 and 31) is considered, particularly as to loss of blood which was different from the two blood vessels of each animal. In two of these animals the coagulation time and bleeding time were the same, but in one animal (Experiment 29) the coagulation time and bleeding time from one vessel were about one sixth of that of the other and the loss of blood was greater. In other words it appears that what favorable results were obtained with the thromboplastins were accidental owing to the tremendous range

of experimental error present in the method. Low levels of blood pressure did not seem to offer any advantage over high levels.

Serum Products.—So far as bleeding time and coagulation time are concerned these were the same in the four animals (Experiments 27, 34, 28 and 37) treated with coagulen and hemostatic serum, but the loss of blood from the arteries in two of the animals was extremely variable. Magalhaes⁶ claims hemostatic action for a hemostatic serum in tubal abortion and cesarean section, but whether this is the same preparation as used by us is not known.

The lack of uniformity in the results and the undecidedness of the method prompted us to abandon further experimentation along this line. Moreover, it is doubtful if any advantage can be secured by the addition of tissue extracts or their active constituents (such as kephalin) to such large wounds already bountifully supplied with tissue juice.

CONCLUSIONS

The extreme variability of results obtained after the application of various thromboplastic agents to dissected femoral arteries bleeding into Scarpa's triangles and the results from untreated vessels under similar conditions indicate that this method of testing these agents is unreliable and unsatisfactory.

If anything, the results indicate the worthlessness of kephalin, coagulen, and hemostatic serum and probably also of the thromboplastins as hemostatics under these conditions.

3. EFFECT OF VARIOUS THROMBOPLASTIC AGENTS ON HEMORRHAGE FROM BONE AND LIVER WOUNDS

Several tests with the different thromboplastic agents were made on a bone wound in the right tibia of a dog, and also on stab wounds of different depths in the liver. The bone wound consisted of an excavation exposing the marrow and was about 1 cm. wide and 4 cm. long. A slow oozing of blood was obtained. Cessation of bleeding and coagulation were observed

6. Magalhaes: Arch. Brasil. de Med. 8:735, 1918.

TABLE 4.—EFFECT OF VARIOUS THROMBOPLASTIC AGENTS
ON HEMORRHAGE FROM BONE AND LIVER WOUNDS
(EXPERIMENT 25; DOG, 14 KG., BLOOD PRES-
SURE LEVEL = 60 MM.)

Bone wound (excavation in right tibia)

Controls, Agents and Con- centration Used	Mode of Applica- tion	Coagulation Time, Minutes	Bleeding Time, Minutes
Normal saline*...	Direct irrigation	2 (soft clot)	2
Thromboplastin; whole (Armour, fresh, 2/17/19)	Direct irrigation; 0.5 c.c.	2¼	2¼ (more blood in wounds)
Normal saline....	Direct irrigation; 0.0 c.c.	2 (soft clot)	2
Thromboplastin; whole (Armour, fresh, 2/17/19)	Direct irrigation; 0.5 c.c.	>3½	>3½ (bleeding un- checked)
Normal saline....	Wiped with cotton pledget every 60 seconds until bleeding ceased		>6 (bleeding un- checked)
Thromboplastin; whole (Armour, fresh, 2/17/19)	Wiped with cotton pledget every 60 seconds until bleeding ceased	>2¾ (soft clot)	>8 (bleeding un- checked)
Normal saline....	Wiped with cotton pledget every 60 seconds until bleeding ceased	3	>8 (bleeding un- checked)
Kephalin, 5 per cent. (fresh pig's, 2/19/19)	Wiped with cotton pledget every 60 seconds until bleeding ceased	3¾ (soft clot)	8 (bleeding un- checked)
Normal saline....	Wiped with cotton pledget every 60 seconds until bleeding ceased	2	>8 (bleeding un- checked)
Coagulen, 3 per cent. (fresh, dry 2/19/19)	Wiped with cotton pledget every 60 seconds until bleeding ceased	5	>8½ (bleeding un- checked)
Hemostatic se- rum (fresh, 2/17/19)	Wiped with cotton pledget every 60 seconds until bleeding ceased	9	>9 (bleeding un- checked)
Epinephrin 1:1000	Wiped with cotton pledget every 60 seconds until bleeding ceased	4½	>9 (bleeding un- checked)

Liver wounds (wiped once every minute with cotton pledget
saturated with agent)

Controls, Agents and Concentrations Used	Depth of Stab, Cm.	Bleeding Time, Minutes
Normal saline	Moderate 0.5	¾
Normal saline	0.5	1½
Thromboplastin, whole (Armour, fresh, 2/17/19) ..	0.5	1¼
Normal saline	1.0	3
Thromboplastin, whole (Armour, fresh, 2/17/19) ..	1.0	1½
Normal saline	1.0	1½
Kephalin, 5 per cent. (fresh, pig's, 2/7/19)	1.0	47 seconds
Normal saline	1.0	1 1/10
Kephalin, 5 per cent. (fresh, pig's, 4/7/19)	1.0	2½
Normal saline	1.0	1¼
Kephalin, 5 per cent. (fresh, pig's, 4/7/19)	1.0	4½
Normal saline	1.0	1½
Coagulen, 3 per cent. (fresh, dry, 2/19/19)	1.0	1½
Coagulen, 3 per cent. (fresh, dry, 2/9/19)	1.0	2½
Hemostatic serum (fresh, 2/17/19)	1.0	1 2/15
Hemostatic serum (fresh, 2/17/19)	1.0	3 1/12
Epinephrin 1:1000	1.0	3 1/12
Epinephrin 1:1000	1.0	2 1/12
Normal saline	1.0	5 7/12

* Normal saline = 0.9 per cent. NaCl. The sign (>) means more than.

by direct inspection. In the majority of tests the agents were applied by saturating a cotton pledget with which the wound was gently swabbed once every minute until the bleeding stopped. At times irrigations were also practiced. Saline was used as the control. The results are presented in Table 4, and indicate no important differences as compared with the controls.

With the bone wounds, bleeding generally tended to continue irrespective of the agent applied; also true of the controls with saline. This is in accordance with the general reputation enjoyed by bone wounds in surgical practice. The clotting of blood, which took place, seemed inadequate to stop the oozing of blood.

The bleeding from stab wounds of the liver, both shallow and deep, stopped promptly, whether treated with saline alone or the thromboplastic agents. The results did not warrant further extension of similar experiments, which are difficult to control properly and are, therefore, unsatisfactory as pointed out in a previous publication by one of us (P. J. H.).

CONCLUSIONS

The applications of the various thromboplastic agents to bone and liver wounds of a dog gave unsatisfactory results as to hemostasis. If anything these were negative and do not support the claims that have been made.

4. THE EFFECT OF SYSTEMIC HEMORRHAGE ON THE COAGULATION TIME OF BLOOD

The various thromboplastic agents and preparations on the market are recommended for use by almost every known method of administration, including the intravenous, for both local and systemic bleeding. Howell¹ has reported favorable effects on blood coagulation after the gastric administration of kephalin to dogs. That is, coagulation time was accelerated, but it was also observed by Howell that bleeding itself tends to hasten coagulation. It was our original intention to test the various thromboplastic agents along this line, but the results obtained with bleeding alone were so discouraging that the attempt was abandoned.

The method consisted of withdrawing blood from the femoral artery, of a morphinized dog into small vials and observing the coagulation time using complete invertability as the end point.

The results presented in Table 5 indicate that bleeding alone markedly and rather promptly shortened the bleeding time in the four animals that were observed.

TABLE 5.—EFFECT OF HEMORRHAGE ON THE COAGULATION TIME OF BLOOD IN DOGS

Number of Experiment	Condition	Number of Blood Sample							
		1	2	3	4	5	6	7	8
		Coagulation Time End of Minutes After Bleeding Started							
13	Normal, 10:20.....	10	..	..	..	..	..	..	..
	Peptonized, 11:10..	>33 (80)	14 (69)	16 (78)	>34 (347)	..	..	..	..
16	Normal, 10:06.....	8	7½ (8)	2 (54)	2 (64)	..	..	..	..
	Peptonized, 11:26½	2 (96)	4 (97)	9 (108)	9 (116)	7 (144)	..	..	..
17	Normal, 9:58.....	9	¾ (38)	¾ (40)	3 (47)	..	..	..	..
	Peptonized, 10:58..	27 (88)	75 (116)	80 (177)	..	..	..	..	..
18	Normal, 10:10.....	3	2½ (16)	3 (22)	¾ (40)	¾ (46)	5/8 (53)	..	..
	Peptonized, 3:01... (23 hrs.)	>1080							
22	Normal, 10:08.....	8	1¾ (10)	1½ (41)	5/8 (56)	1½ (86)	1¾ (102)	2¼ (113)	2¼ (140)

* Figures enclosed by parentheses represent number of minutes after withdrawal of blood was begun. The left femoral artery was used in all experiments.

The sign (>) means more than.

Taking the experiments individually it is seen that coagulation time was hastened by 75 per cent. in fifty-four minutes, being unchanged at the end of one hour and four minutes after blood was first drawn in Experiment 16. In Experiment 17, coagulation time was shortened by 92 per cent. in thirty-eight minutes, 90 per cent. in forty minutes and about 67 per cent. at the end of forty-seven minutes after bleeding was started. In Experiment 18, coagulation time was shortened by

75 per cent. in forty minutes and 70 per cent. at the end of fifty-three minutes after bleeding was started. In Experiment 22 the coagulation time was shortened by 78 per cent. at the end of ten minutes, 90 per cent. at the end of fifty-six minutes and 72 per cent. at end of 140 minutes after the blood was first drawn. From this it appears that any favorable claims which may be made for the systemic administration of thromboplastic or other agents on the coagulation time of blood obtained by repeated bleedings at intervals from an artery must be regarded with suspicion. We considered that the investigation of thromboplastic agents along this line was entirely precluded on the basis of our results obtained with normal and untreated animals.

Some of the animals were later peptonized with the idea of obtaining a noncoagulable blood which could be drawn over considerable periods of time without the natural tendency to hasten clotting time from bleeding alone. The blood of such animals was temporarily rendered noncoagulable although variable in the four animals (Table 5) that were tried, but all of these suffered a fatal fall of blood pressure and died shortly after the injection of peptone.

Such experiments could hardly be expected to show any more than peptonized plasma or blood *in vitro*, data concerning which are presented in the first paper⁴ of this series.

CONCLUSIONS

Bleeding at intervals from arteries of normal animals markedly (by 72 to 90 per cent.) and promptly (ten to forty-seven minutes) shortens the coagulation time of blood, rendering it impossible to arrive at any definite conclusion concerning the thromboplastic activity of agents administered systemically.

5. THROMBOPLASTIC AGENTS IN HEMOPHILIA

Logically it appears that kephalin and similar thromboplastic agents are not therapeutically indicated in any condition, including hemophilia, unless the tissues are deficient in the thromboplastic agent, or the blood

contains an excess of some of the constituents such as antithrombin which interferes with clotting and on which kephalin is considered to act. According to Howell,¹ an excess of antiprothrombin in hemophilia is considered possible. Neutralization of this by kephalin should accelerate the coagulation time, and, therefore, furnish a rational therapeutic remedy. Favorable results are reported to have been observed by Howell,¹ also by Lucas and Hurwitz,⁷ and others. Lowenburg and Rubenstone⁸ observed acceleration of clotting of hemophilic blood with thromboplastin (Squibb). According to others,⁹ the etiology of hemophilia is different; and equally good or bad hemostatic results are reported by the use of the agents based on an entirely different conception of blood coagulation. Thus it appears that the inharmonious views and contradictory reports on this subject render any conclusions insecure and dubious. This is due in part to the relatively few cases of hemophilia available for definite observations, the impossibility of securing reliable controls, the uncontrolled clinical observations and enthusiastic reception accorded to any new remedy. These tendencies are too prevalent in the few reports in the literature on the therapeutics of hemophilia. The literature of the manufacturers of this class of preparations generally credits the thromboplastic agents with specificity in hemophilia and other bleeding conditions.

During the course of our work there was an opportunity to administer kephalin to a patient with hemophilia. Although it might be inferred that the use of kephalin in this patient was beneficial, it is not believed that such a conclusion is justified, since bleeding stopped spontaneously on occasions before the administration of kephalin and conceivably might have stopped again in spite of the administration of kephalin during the long attack of intestinal bleeding. The abbreviated protocol of the patient follows:

7. Hurwitz and Lucas: *Arch. Int. Med.* **17**: 543, 1916.

8. Lowenburg and Rubenstone: *J. A. M. A.* **71**: 1196, 1918.

9. Minot and Lee: *Arch. Int. Med.* **18**: 474, 1916; Hess: *Arch. Int. Med.* **17**: 203, 1916. Ducceschi: *Arch. ital. biol.* **64**: 341.

Patient.—H. H., boy; aged 14 years.

Past History.—Sick three months; came into Lakeside Hospital with hemarthria; previously known to be hemophilic. Bled recently as a result of tonsillectomy. Bled profusely all night from tonsils. There were hemorrhages also in knee joints. Sustained abrasion of elbow joint in operating room and bled continuously for several days. Suture of abrasion was resorted to and finally bleeding stopped spontaneously. Boy was transfused at the time of severe tonsillar hemorrhage. Bleeding from transfusion incision was difficult to stop, but eventually ceased. Prolonged coagulation time and bleeding time were demonstrated in the hospital. Diagnosis, hemophilia by Prof. C. F. Hoover.

Present Illness.—Feb. 25, 1919, the boy was at Rainbow Cottage with bleeding from bowels. Fresh macroscopic blood was present in stools, which were rather liquid for the last eight days. Blood was continuously present; benzidin was positive and blood cells were present microscopically. Boy was very anemic. The following medication was given by Dr. F. Blankenhorn, using fresh sheep kephalin prepared in the laboratory and which markedly accelerated clotting of blood and oxalate plasma.

February 26. 0.5 gram kephalin per os 7:00 a. m.; 0.5 gram kephalin per os 7:00 p. m.

February 27. 1 gram kephalin same as on February 26.

February 28. 1 gram kephalin same as on February 26.

March 1. 1 gram kephalin at 7:30 a. m.

Bleeding time (right ear)¹⁰ = 21 minutes.

Coagulation time (right ear) = 18 minutes.

Administration of kephalin stopped. Total given 4 grams.

March 2. Still claimed to be free from blood.

March 4. No blood in stool microscopically.

March 5. Benzidine test positive; no microscopic blood in stool.

March 6. No blood in stool microscopically (two smears).

March 8. No blood in stool microscopically.

March 9. No blood in stool microscopically.

10. Duke's (Duke, W. W.: J. A. M. A. **55**: 1185, 1910) methods for obtaining coagulation time and bleeding time were used. The normal range of coagulation time according to Duke is from five to seven minutes; of bleeding time, one to three minutes. The results obtained indicate definite prolongation of both the bleeding time and coagulation time. The patient was so anemic that the withdrawal of larger quantities of blood and venipuncture necessary in the Morawitz method were precluded.

Right ear. Bleeding time = 9 minutes. Clotting time = 14 minutes.

Left ear. Bleeding time = 11 minutes. Clotting time = 16 minutes.

During the last four months the patient has remained well.

CONCLUSIONS

The administration of 4 gm. of kephalin by mouth to a patient with hemophilia and suffering with a troublesome intestinal hemorrhage was followed by prompt stoppage of the hemorrhage and shortening of the coagulation time and bleeding time of ear blood. This is not interpreted as due necessarily to kephalin, since bleeding in this patient was known to have stopped spontaneously before the treatment, illustrating the necessity of exercising the greatest care in interpreting data of this sort.

III. SUMMARY

(The numbers refer to the sections in the text)

1. The hemostatic effects of the thromboplastic agents tested on superficial hemorrhage from the dog's pad, although limited and variable, in general tend to agree with their power to accelerate coagulation of blood and plasma *in vitro*. Accordingly the thromboplastins and kephalin are among the most active; followed by saline and coagulen and hemostatic serum as either inactive or doubtful.

2. The extreme variability of results obtained after the application of various thromboplastic agents to dissected femoral arteries bleeding into Scarpa's triangles and the results from untreated vessels under similar conditions indicate that this method of testing these agents is unreliable and unsatisfactory. If anything, the results indicate the worthlessness of kephalin, coagulen, hemostatic serum and probably also the thromboplastins, as hemostatics under these conditions.

3. The application of various thromboplastic agents to bone and liver wounds of a dog gave unsatisfactory results. If anything, these were negative and do not support the claims that have been made for them.

4. Hemorrhage per se at intervals from arteries of dog markedly (by 72 to 90 per cent.) and promptly (ten to forty-seven minutes) shortens the coagulation time of blood, rendering it impossible to arrive at any definite conclusions concerning the thromboplastic activity of agents administered systemically.

5. The administration of 4 gm. of kephalin by mouth to a patient with hemophilia and suffering with a troublesome intestinal hemorrhage was followed by prompt stoppage of the bleeding with shortening of the coagulation time and bleeding time of ear blood. This is not interpreted as due necessarily to kephalin, since bleeding in this patient was known to have stopped spontaneously before the treatment, illustrating the necessity of exercising the greatest care in interpreting data of this sort.

THE SOLUBILITY OF INTESTINAL IPECAC PREPARATIONS *

TORALD SOLLMANN, M.D.

CLEVELAND

(From The Journal A. M. A., Oct. 11, 1919, p. 1125)

The reputed value of ipecac against intestinal amebas was originally based on its oral administration. Its nauseant and emetic actions, however, constituted serious drawbacks to its use. The coating of pills with salol is not always satisfactory, although, with due care, it appears to be quite feasible.¹ The hypodermic injection of emetin hydrochlorid avoids the local actions; but used thus, it often fails to rid the patient of amebas, especially if he is a chronic carrier. Attempts have therefore been made to solve the problem by the use of emetin compounds that, while insoluble in the stomach, would be soluble in the intestine. The basis of these attempts lies in the difference between the reaction of the stomach and that of the intestine.

EMETIN BISMUTH IODID

Emetin bismuth iodid is described in New and Non-official Remedies and a further description is found in the report of the Council on Pharmacy and Chemistry for 1918.² Emetin in this form is only slightly soluble in water and dilute acids, but dissolves quite freely in 1 per cent. sodium bicarbonate. It is evidently somewhat soluble in the stomach, and accordingly produces some digestive disturbances, although these are much less severe than with ipecac proper.²

* From the Department of Pharmacology of the Western Reserve University School of Medicine.

* The investigation was supported by a grant from the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

1. Simon, S. K.: Comparative Value of Ipecac and Its Alkaloids in Treatment of Intestinal Endamebiasis, *J. A. M. A.* **71**:2042 (Dec. 21) 1918.

2. Report on Emetin Bismuth Iodid, *J. A. M. A.* **71**:2013 (Dec. 21) 1918.

ALCRESTA IPECAC

Alcresta ipecac is an adsorption product of ipecac and fuller's earth (hydrous aluminum silicate) especially prepared according to the method of J. U. Lloyd, who discovered that practically all alkaloidal salts are adsorbed by this powder and are thereby rendered insoluble in neutral or acidulated water.³ This finding was confirmed by Gordin and Kaplan.⁴ The alkaloids can again be dissolved by treatment with chloroform in the presence of fairly strong alkalis.

Tablets of alcresta ipecac were described⁵ in N. N. R., 1917, on the basis of the claims of the manufacturers that their alkaloids "are physiologically inert as long as they remain within the stomach, and are rendered active when set free in the alkaline media of the intestine." This was interpreted to mean that they are insoluble in the reaction of the stomach, but become soluble in the intestine.

RELATIVE SOLUBILITY OF EMETIN BISMUTH IODID
AND ALCRESTA IPECAC IN DIGESTIVE SOLVENTS

An attempt was made to demonstrate the assumed behavior of these preparations in the stomach and the intestine. The gastric reaction was represented by 0.2 per cent. hydrochloric acid; the intestinal reaction by 1 per cent. sodium bicarbonate.

The experiments were made as follows: The powders were shaken thoroughly with the solvent, filtered, and the filtrate acidulated with hydrochloric acid, if necessary, and tested with mercuric potassium iodid (Mayer's reagent). Solution of the alkaloids is indicated by the occurrence of a turbidity or precipitate when the reagent is added.

The details are shown in Table 1. Briefly, the experiments showed that emetin bismuth iodid is almost but not quite insoluble in the acidity of gastric juice, and

3. Lloyd, J. U.: *J. Am. Pharm. A.* **3**: 625, 1914; **5**: 381, 1916.

4. Gordin and Kaplan: *J. Am. Pharm. A.* **3**: 627, 1914.

5. They were dropped from N. N. R. during that year because of "exaggerated, misleading and harmful" therapeutic claims (Report on Alcresta Ipecac, *J. A. M. A.* **69**: 1373 [Oct. 20] 1917.)

that it is freely soluble in the alkalinity of the intestinal juice. This is as was expected. The adsorption product of ipecac and Lloyd's reagent, however, was insoluble not only in the acid medium but also in the bicarbonate. Charcoal, incidentally, did not adsorb the alkaloids effectively even from acid solution.

The insolubility of the adsorption product in bicarbonate seemed so important a contradiction to the clinical use of the product that the investigation was extended to the commercial tablets, and was modified in various directions.

TABLE 1.—PRELIMINARY COMPARISON OF THE SOLUBILITY OF EMETIN BISMUTH IODID, AND OF IPECAC ADSORPTION PRODUCTS

	Solubility in 0.2 % Hydro- chloric Acid	Solubility in 1 % Sodium Bicarbonate
Emetin bismuth iodid (0.05 gm. + 10 c.c. of solvent)	Slight	Very considerable
Ipecac—Lloyd's reagent (2 c.c. 20 % tincture, 10 c.c. 0.2 % hydrochloric acid, 1 gm. Lloyd's reagent)	None	Very slight
Ipecac—animal charcoal (as preced- ing, except that the charcoal is used in place of Lloyd's reagent)	Considerable	Very slight

SOLUBILITY OF ALCRESTA IPECAC IN ALKALINE SOLVENTS

Three specimens of alcresta ipecac tablets were used in these experiments. One specimen was submitted by the manufacturers in 1915; the others were purchased recently in Chicago and Cleveland. The statements that follow may be regarded as holding true of all three specimens, unless exceptions are mentioned. Several specimens of emetin hydrochlorid were used for purposes of comparison.

It may be mentioned that 0.2 per cent. and 0.5 per cent. hydrochloric acid fail to dissolve any of the alkaloid, even on incubation; that is, the filtrates are unchanged by the mercuric potassium iodid reagent.

SOLUBILITY IN SODIUM BICARBONATE

The alcresta ipecac samples were powdered and mixed with from 0.5 to 1 per cent sodium bicarbonate, from 10 to 100 c.c. per tablet, and filtered either at once or after being allowed to stand up to four days.

The filtrate (which was always distinctly alkaline to litmus) was then acidulated with hydrochloric acid and tested for dissolved alkaloids by the mercuric potassium iodid reagent. The results (Table 2) were absolutely negative, with the possible exception of the specimens that had stood four days, in which a part of the bicarbonate may have been converted into carbonate.

TABLE 2.—SOLUTION OF IPECAC ALKALOIDS IN SODIUM BICARBONATE

Preparation	Dilution	Concentration of Sodium Bicarbonate	Length of Contact	Result with Merc. Pot. Iod.
Emetin HCl.....	1:100,000	1%	Short	Marked turbidity
Emetin HCl.....	1:100,000	Supersaturated	Overnight	Marked turbidity
Alcresta tablets. Samples	1 tablet per c.c.			
All three.....	100	1%	1 day	Clear
Cleveland.....	10	0.5%	1 day	Clear
Cleveland.....	10	1%	Short	Clear
Cleveland.....	10	1%	1 day at 39 C.	Clear
Cleveland.....	10	1% + 0.5% bilin	1 day at 39 C.	Clear
All three.....	10	1%	4 days	No change at once, but slight turbidity on standing

Theoretical objection could be made to these experiments on the assumption, first, that emetin is liberated, but is insoluble in bicarbonates, and second, that the reagent is too insensitive to reveal small quantities of dissolved emetin.

Neither assumption is tenable. As for the solubility of emetin in bicarbonate: the experiments of Table 2 show that emetin is sufficiently soluble in bicarbonate for the solution to give a marked precipitate with Mayer's reagent. In fact, a 1:10,000 solution of emetin is not precipitated by the addition of sodium bicarbonate; whereas sodium carbonate or hydrate pro-

duce turbidity. Stronger solutions were not tried. The delicacy of Mayer's reagent is such that a drop added to 5 c.c. of the emetin hydrochlorid solutions gives turbidity with a 1:100,000 solution and precipitation with a 1:10,000 solution. It should also be noted that the addition of bile salts does not increase the solubility of the alcresta ipecac in bicarbonate solution.

There is a further possibility, namely, that the emetin is liberated by the bicarbonate from the adsorbent, but for some reason fails to dissolve. This assumption is vague, and even if tenable, there would seem to be no practical advantages in "liberating" an alkaloid, except for the purpose of dissolving it. However, to dispose of the suggestion, and to check up the results, the alcresta ipecac-bicarbonate mixture or the residue

TABLE 3.—ETHER EXTRACTS OF ALCRESTA IPECAC-BICARBONATE MIXTURES

Alcresta Tablets (1 per test)	Bicar- bonate solution (c.c.)	Concen- tration of Bicar- bonate	Length of Contact	Ether Extract of Mixture or Residue	Result with Mayer's Reagent
All three.....	10	1%	4 days	Residue	0
All three.....	10	1%	Short	Mixture	0
All three.....	100	1%	1 day	Mixture	0
Cleveland.....	6	0.5%	1 day	Residue	0

remaining after the filtration of the bicarbonate solution was extracted with ether. The ether was separated, filtered and evaporated, and the residue taken up with dilute acid and tested with Mayer's reagent. The results (Table 3) are uniformly negative and confirm that the emetin of the alcresta ipecac-bicarbonate mixtures is also insoluble in ether.

SOLUBILITY OF ALCRESTA IPECAC IN STRONGER ALKALIS

It appeared from these experiments that the assumption of the intestinal liberation of emetin from alcresta ipecac is untenable. It was therefore thought that a test of the effect of somewhat stronger alkalis on alcresta ipecac might prove instructive. For this purpose, the residues remaining after the fruitless extrac-

tion with bicarbonate were treated successively with sodium carbonate, added to 0.5 or 1 per cent., and then with sodium hydroxid, added to 0.5 per cent. The mixtures were extracted at each stage with ether, and this was filtered, evaporated, acidulated and tested as usual. The results are shown in Table 4. They indicate that the emetin, although not liberated by the sodium bicarbonate, is indeed liberated to some extent by sodium carbonate, and much more by sodium hydroxid.

PRECIPITATION OF ALKALINE EMETIN BY
LLOYD'S REAGENT

The assumption that alkaline emetin is soluble in the presence of Lloyd's reagent was further tested directly by shaking alkaline solutions of emetin with Lloyd's

TABLE 4.—SOLUBILITY OF ALCRESTA IPECAC IN STRONGER
ALKALIS *

Aleresta Tablets	Sodium Bicarbonate, 1%	Sodium Carbonate 0.5 to 1% to Residue	Sodium Hydroxid 0.5% to Residue
All three speci- mens 1:10 c.c.	Clear	Slightly turbid	Large precipitate
1915	Clear	Very slightly turbid	
Chicago	Clear	Clear	
Cleveland	Clear	Considerably turbid	

* Reaction of ethereal extracts of the solutions as stated in the columns, with Mayer's reagent.

reagent. It was found that this removed the alkaloid just as completely as from acid mediums, and rendered it insoluble, not only in watery solutions, but even in ether.

The experiments were made by adding alkalis to a 1:100,000 solution of emetin, till these contained 1 per cent. respectively of sodium bicarbonate, sodium carbonate and sodium hydroxid.

The solution in bicarbonate was clear; that in carbonate had a slight precipitate; that in hydroxid was slightly turbid, with a rose tinge.

Half of each sample was shaken with Lloyd's reagent. The reaction remained alkaline to litmus in every case. Samples of the mixtures were then filtered,

acidulated and tested with Mayer's reagent. The remainder of each mixture was then shaken out with ether, the ether filtered, evaporated, acidulated and tested with Mayer's reagent.

The results, shown in Table 5, prove clearly that Lloyd's reagent binds emetin in alkaline solution.

ACTION OF IPECAC ALKALOIDS WITHOUT SOLUTION

When the insolubility of the alcresta ipecac alkaloids became apparent, this information was communicated to the manufacturers. In their reply they conceded this insolubility and added: "We do not state that the ipecac alkaloids are soluble in a solution of the alkalinity of intestine, but only that they exert their physio-

TABLE 5.—LLOYD'S REAGENT OF ALKALINE SOLUTIONS OF EMETIN *

	Sodium Bicarbonate Direct Test	Sodium Carbonate		Sodium Hydroxid	
		Direct	Ether Ext.	Direct	Ether
Mixtures without Lloyd's reagent	Marked turbid- ity	Turbid	Consider- ably tur- bid	Turbid	Consider- ably tur- bid
Mixtures with Lloyd's reagent	Clear	Clear	Clear	Clear	Very slightly turbid

* Mayer's test of dissolved alkaloid applied to the acidulated filtrates.

logical effect in the intestine and, as you of course understand, solubility in water is not necessary in order to bring about absorption from the intestinal tract."

These statements cannot be accepted. I doubt very much whether any substance can be absorbed, undissolved, from the intestine. Solubility in water, of course, has no bearing on the case, for I have aimed to imitate the solubility characters of the intestinal fluid.

It appears equally doubtful whether, undissolved, insoluble and adsorbed emetin can kill amebas. I tried to approach this problem by testing the toxicity to earthworms; but the low toxicity of emetin for these animals (they survive 1:10,000) rendered this impracticable.

The pharmacologic evidence indicates quite strongly that the emetin-alcresta ipecac is quite inactive in the digestive tract of dogs. Fantus⁶ found that a doubled fatal dose of ipecac produced no effects of any kind when mixed with fuller's earth. Eggleston and Hatcher⁷ likewise found no effects whatever from doses of alcresta ipecac that would have been fatal had a pure ipecac been administered.

This is as far as the positive evidence at present extends. Ordinarily, it would not be expected that a substance that is quite insoluble in the intestine, and that has completely lost its effects on the intestinal cells, which are ordinarily very sensitive to it, should still be effective on amebas, which are ordinarily affected only by high concentrations. Such a contradiction should demand a very careful and critical reexamination of the clinical evidence. As I am not in a position to undertake this, I must leave the subject in this incomplete state.

CONCLUSIONS

Emetin bismuth iodid is only slightly soluble in fluids of the acidity of the stomach, but freely soluble in fluids of the alkalinity of the intestine. These findings agree with the clinical data, namely, that it is an effective amebicide, but is not altogether devoid of gastrointestinal irritation.

The alkaloids of alcresta ipecac are entirely insoluble, both in acid and alkaline solutions of physiologic concentration. The solubility is not improved by the addition of bile salts or of albumin.

The pharmacologic literature of alcresta ipecac confirms that the emetin remains entirely inactive.

6. Fantus, Bernard: Fuller's Earth; Its Adsorptive Power and Its Antidotal Value for Alkaloids, *J. A. M. A.* **64**: 1838 (May 29) 1915.

7. Eggleston and Hatcher: *J. Pharmacol. & Exper. Therap.* **7**: 241, 1915.

CORRECTION.—Through an error the name of Torald Sollmann, M.D., Cleveland, is given as one of the authors of "The Pharmacology of the Local Anesthetics." The authors are Cary Eggleston, M.D., and Robert A. Hatcher, M.D.

THE PHARMACOLOGY OF THE LOCAL ANESTHETICS*

TORALD SOLLMANN, M.D.

CLEVELAND

CARY EGGLESTON, M.D.

AND

ROBERT A. HATCHER, M.D.

NEW YORK

(From The Journal A. M. A., Oct. 25, 1919, p. 1256)

Acute intoxication in man from the use of the local anesthetics is far more common than is indicated by the number of recorded cases. We undertook to investigate the causes of such intoxication, and sought to find means of avoiding it, or combating it when it arises. This investigation has included a study of the literature of the more important cases of human intoxication, and the performance of over 300 experiments on cats. The details of this work are published elsewhere,¹ the present communication being limited to a short discussion of the more important facts brought to light, especially with reference to their bearing on the clinical problem.

Nine of the local anesthetics were studied, and the maximum toxicity of each was determined by rapid intravenous injection into cats. The drugs used, the fatal dose of each, the concentrations of the solutions employed, and the relative toxicities of the drugs are shown in the accompanying chart. From the chart it is seen that cocain is about intermediate in the group

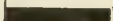
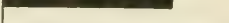
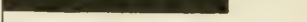
* From the Department of Pharmacology, Cornell University Medical College.

* Read before the Section on Pharmacology and Therapeutics at the Seventieth Annual Session of the American Medical Association, Atlantic City, N. J., June, 1919.

* Part of the expense of this research has been defrayed by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

1. J. Pharmacol. & Exper. Therap. **13**, 1919.

with reference to its maximal toxicity for the cat. These relative toxicities, however, apply only for single doses injected rapidly into the blood stream. When large fractions of the minimal fatal doses are injected intravenously at intervals of from fifteen to twenty minutes, or when relatively dilute solutions of the drugs are injected slowly and nearly continuously, the several drugs can be divided into two groups based on the difference in their rates of elimination. Group 1 includes alypin, apothecin, beta-eucain, nirvanin, procain (novocain), stovain and tropacocain, all of which are rapidly eliminated, so that several times the minimal fatal dose

		Mgm. x kgm. 40 - 45	Solution, Per cent. 5 - 20
Procain			
Nirvanin		30 - 35	10
Stovain		25 - 30	10
Tropacocain		18 - 22	10
Apothecin		20	5 - 10
Cocain		15	5 - 10
Beta-Eucain		10 - 12.5	5
Alypin and Holocain		10	5 - 10 1 - 2

Relative toxicity and range of concentration of solutions for the several local anesthetics; fatal doses in milligrams per kilogram.

of any of these can be injected in the ways just mentioned in periods of one or two hours without causing death of the animal. Group 2 includes cocain and holocain, which are much less rapidly eliminated, and which therefore cause death in much smaller total doses when given as described.

When injected subcutaneously into cats, the local anesthetics can again be divided into the same two groups, depending on their rates of elimination. All are apparently absorbed rapidly, but those of Group 2 are much more toxic by subcutaneous injection than those of Group 1 because their elimination is not rapid

enough to keep pace with their absorption. Five or more times the minimal fatal vein dose of any of the members of Group 1 can be injected subcutaneously without causing death, while less than four times the fatal vein doses of those of Group 2 prove fatal.

When absorption from the subcutaneous tissues is delayed by the simultaneous injection of epinephrin, the difference between the two groups is emphasized, and the toxicity of the members of the first group is reduced far more than that of the members of Group 2. The elimination of members of Group 1 proceeds at the rate of at least one fatal vein dose every twenty minutes, while not all of a subcutaneous dose of cocaine, one and one-third times that fatal by vein, may be eliminated in twenty-four hours or more.

The elimination of all of the local anesthetics is accomplished almost entirely by their destruction in the liver, as can be demonstrated by perfusion of the excised, surviving organ with diluted blood containing large quantities of the drugs in solution, and subsequent determination of the amount remaining in the perfused fluid.

Various efforts were made to influence the toxicity of the local anesthetics, including the administration of atropin or caffeine prior to the intravenous injection of the anesthetics; the addition of sodium bicarbonate to the solution of the anesthetic just before intravenous injection, to liberate the base; the production of nervous exhaustion in the cat; severe acute hemorrhage by removal of one fourth of the cat's total blood; and the production of narcosis by the administration of hydrated chloral. None of these measures had any influence on the cat's susceptibility to the intravenous injection of any of the anesthetics except acute hemorrhage and narcosis by chloral. Both of these caused some increase in the cat's susceptibility, as shown by the fact that often it succumbed to doses smaller than the average fatal dose. This increased susceptibility is accounted for by the fact that both these measures interfere with the destruction of the anesthetics in the liver by reduction in the blood pre-

sure and impairment of the circulation. The resistance of both the heart and the respiratory center is also probably reduced by the impaired circulation.

The symptoms produced in cats by toxic doses of the various local anesthetics are essentially alike, and they are also essentially the same as those observed in man. All of these drugs kill by simultaneous paralysis of the heart and the respiratory center. We therefore tried artificial respiration in cats as a resuscitative measure, with and without cardiac massage, but it proved ineffective, even when combined with massage of the heart.

The employment of artificial respiration in combination with stimulation of the heart by the immediate intravenous injection of epinephrin, however, enabled us to resuscitate the majority of cats after the rapid intravenous injection of doses of the local anesthetics up to twice the average fatal dose. Since the heart had stopped in most of the cats, it was necessary to practice massage of the heart in order to bring the epinephrin into contact with it. The success of this method of resuscitation depends on the rapid destruction of the local anesthetics, whereby the excess administered is promptly eliminated if the circulation can be maintained for only a few minutes by the stimulation of the heart with epinephrin. But even with such stimulation, and when it is evidently recovering, the animal will die of respiratory paralysis unless the center is supported for a few minutes by artificial respiration.

The success of the preceding measures, in which the brief stimulation of the heart played the most important part, led us to try the effects of previous stimulation of the heart by the administration of a dose of ouabain (so-called crystalline strophanthin). We then administered doses of the local anesthetics up to twice the average fatal dose, and employed artificial respiration to tide over the period of respiratory paralysis. By these measures, cats usually survived doses of the anesthetics up to one and three quarter times the average fatal dose.

It is evident, therefore, that if the circulation and respiration can be maintained for even a few minutes, the rapid destruction of the local anesthetics by the liver will care for amounts considerably in excess of those which are usually fatal. The close analogies between the behavior of man and cats to toxic doses of the local anesthetics, and the fact that man also recovers rapidly from the effect of nonfatal doses, suggest that he also accomplishes their elimination or destruction in his liver, and it seems highly probable that the use of epinephrin and artificial respiration will prove effective as resuscitative measures in many cases of acute poisoning in man. It is, however, to be remembered that serious intoxication has been seen in man following extremely small doses of the local anesthetics, suggesting the occasional existence of a marked degree of hypersusceptibility; and in such cases doses which are relatively small, as compared with the average commonly employed, may actually be several times the minimum which would be fatal in the particular individual, so that even the most effective resuscitative measures may well fail under such circumstances.

While cardiac massage cannot be carried out so efficiently in man as in the cat, it can be performed sufficiently to cause the greater part of an intravenous injection of epinephrin to reach the heart; and several satisfactory methods are available for carrying on artificial respiration in man. These measures, also, are all such as can be applied without loss of time under most conditions, and they certainly seem to be the most effective means at our disposal for saving life after the development of symptoms of acute intoxication by the local anesthetics. Since acute poisoning in man occurs when least expected, those who frequently employ these local anesthetics should always be prepared to apply the three resuscitative measures of intravenous infusion of epinephrin, artificial respiration, and cardiac massage. All three should be used in combination, since no one alone is effective, except in rare instances.

Finally, in order to diminish the likelihood of intoxication from the subcutaneous injection of the local anesthetics — especially those of Group 1 — in man, epinephrin should be added to their solution as a routine, because by delaying their absorption it renders it more probable that the destruction by the liver can keep the amount present in the circulation at any one time at a point below that sufficient to cause intoxication. The use of epinephrin also has the further advantages of prolonging the anesthetic action of a given quantity of the drug and of reducing the amount required for anesthesia by permitting a larger proportion of the dose injected to remain in contact with the tissues to be anesthetized, and by maintaining the contact for a longer period of time than when the drug is injected alone.

414 East Twenty-Sixth Street.

ANAPHYLACTOID PHENOMENA FROM THROMBOPLASTIC AGENTS *

PAUL J. HANZLIK, HOWARD T. KARSNER
AND JOSEPH FETTERMAN

(From *The Journal of Pharmacology and Experimental Therapeutics*,
November, 1919, p. 229)

The thromboplastic agents which have been discussed in preceding papers¹ may be placed into two groups according to the absence or presence of precipitable protein. The results of the tests for protein are presented in Table 1, and the following tabulation is based on these.

<i>Protein absent</i>	<i>Protein present</i>
Kephalin (fresh; prepared in laboratories)	Hemostatic serum (Parke, Davis Company)
Coagulen (Ciba)	Thromboplastin (Squibb)
Ciagulen solution (Ciba)	Thromboplastin (Armour)
Kephalin (Armour)	

Accordingly, those preparations containing protein might be expected to produce symptoms resembling anaphylaxis when injected intravenously. The literature of the manufacturers gives the contrary impression, but Lee and Minot² have stated that such products as coagulen, etc., may be dangerous when injected.

The proposition was tested by making intravenous and subcutaneous injections into healthy unsensitized guinea-pigs, using salin, and triclesol (0.3 per cent.), which is used as a common preservative for one of these agents, as controls. The intravenous injections were made slowly into either the left or right jugular veins in animals previously lightly etherized for incision of the skin only. The symptoms after injection were

* From the Laboratories of Pharmacology and of Pathology, School of Medicine Western Reserve University, Cleveland.

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1. Hanzlik and Weidenthal: *J. Pharm. Exper. Therap.* **14**: 81, 1919; **14**: 115, 1919.

2. Lee and Minot: *Clev. Med. J.* **16**: 65, 1917.

TABLE 2.—ANAPHYLACTOID PHENOMENA FROM THROMBOPLASTIC AGENT INTRAVENOUSLY AND SUBCUTANEOUSLY

No. of Experiment	Wt. of Guinea-Pig, Gm.	Volume of Solution Injected	Dosage per Gm. of Animal (Mg. or C.c.)	Symptoms	Fate of Guinea-Pig	Killed End of Min.	Necropsy	Microscopic Examination of Lungs
Thromboplastin (Squibb, fresh)								
18	260	3.0 c.c.	0.015 c.c.	Convulsions; relaxation of sphincters; cyanosis; depression	Fatal in 7 min.	..	Lungs collapsed; cardiac dilatation, general abdominal congestion, pneumonia	Moderate distention, marked congestion, and marked thrombosis
66	360	1.0 c.c.	0.0028 c.c.	Dyspnea; increased respiration; jerky spasms of neck	Recovery	45	Slight distention of lungs; marked pulmonary hemorrhages; marked cardiac dilatation; slight abdominal congestion	Slight distention, marked congestion, slight thrombosis
72	390	0.5 c.c. (subcutaneously)	0.0013 c.c.	Restlessness; slight dyspnea; increased respiration	Recovery	32	Lungs distended and congested; heart dilated, slight abdominal congestion	Marked distention, marked congestion, marked hemorrhage, conglutination thrombi
73	430	0.5 c.c. (subcutaneously)	0.001 c.c.	Deep respiration; no effort	Recovery	37	Marked distention of lungs and marked hemorrhages; cardiac dilatation; slight abdominal congestion	Moderate distention, marked congestion, hemorrhage, conglutination thrombi
Thromboplastin (Armour, fresh)								
64	350	1.0 c.c.	0.003 c.c.	Some dyspnea; increased respiration; ruffling of hair	Recovery	50	Marked distention of and hemorrhage in lungs; slight cardiac dilatation; moderate abdominal congestion	Marked congestion
78	215	1.5 c.c.	0.007 c.c.	Dyspnea marked; increased respiratory rate	Recovery	45	Marked distention of lungs; pulmonary hemorrhages; slight cardiac dilatation; abdominal congestion	Moderate distention, marked congestion, marked hemorrhage

33	£75	3.0 c.c.	0.32 mg.	(Congulen (dry, fresh, 3 per cent.) Dyspnea; increased respiration	Recovery 35	Marked inflation of lungs; pulmonary hemorrhages; heart slightly dilated, no abdominal congestion	Moderate congestion
41	220	3.0 c.c. (ampoule solution)	0.45 mg.	Dyspnea; marked increase of respiration; restlessness	Recovery 30	Marked inflation of lungs; pulmonary hemorrhages; cardiac dilatation, no abdominal congestion	Marked distention, marked congestion, moderate hemorrhage, thrombosis
65	420	3.0 c.c. (ampoule)	0.21 mg.	Dyspnea; respiration slowed; restlessness; spasms	Recovery 46	Slight distention of lungs; marked pulmonary hemorrhages; distinct abdominal congestion; heart markedly dilated	Moderate distention, marked congestion, marked thrombosis
67	400	0.5 c.c. (dry-fresh)	0.038 mg.	Marked dyspnea; increased respiration; restlessness	Recovery 40	Lungs moderately distended and congested; no hemorrhages; marked cardiac dilatation; slight abdominal congestion	Slight distention, moderate congestion, fine conglutination thrombi
69	370	0.5 c.c. (ampoule)	0.041 mg.	Marked dyspnea; increased respiration	Recovery 36	Moderate distention of lungs; pulmonary congestion and hemorrhages; cardiac dilatation; moderate abdominal congestion	Marked congestion
71	430	1.0 c.c. (ampoule)	0.07 mg.	Moderate dyspnea; increased respiration	Recovery 33	Lungs moderately distended and markedly congested; pulmonary hemorrhages; moderate cardiac dilatation; abdominal congestion	Marked congestion
75	320	3.0 c.c. (subcutaneous)	0.28 mg.	Dyspnea; increased respiration; restlessness	Recovery 48	Slight distention of lungs; pulmonary congestion and hemorrhages; marked cardiac dilatation; slight abdominal congestion	Moderate distention, marked congestion, marked hemorrhage

TABLE 2.—ANAPHYLACTOID PHENOMENA FROM THROMBOPLASTIC AGENT INTRAVENOUSLY AND SUBCUTANEOUSLY—Cont.

No. of Experiment	Wt. of Guinea-pig, Gm.	Volume of Solution Injected	Dosage per Gm. of Animal (Mg. or C.c.)	Symptoms	Fate of Guinea-Pig. End of Min.	Necropsy	Microscopic Examination of Lungs
79	350	3.0 c.c. (subcutaneously) ampule	0.26 mg.	Dyspnea; increased respiration Coagulen (dry, fresh, 3 per cent.)—Continued	Recovery 68	Lungs distended; marked pulmonary hemorrhage and congestion; cardiac dilatation; slight abdominal congestion	Marked congestion
26	185	3.0 c.c.	0.016 c.c.	Hemostatic serum (fresh; Parke, Davis & Company) Depression; marked dyspnea; increased respiration; convulsions; shivering	Recovery 38	Lungs collapsed and hemorrhagic; cardiac dilatation, pneumonia, general abdominal congestion	Moderate distention, moderate congestion, marked hemorrhage, thrombosis and edema
68	340	2.0 c.c.	0.006 c.c.	Marked dyspnea; increased respiration; restlessness	Recovery 37	Lungs about collapsed; marked pulmonary congestion; slight cardiac dilatation; abdominal congestion	Slight distention; marked congestion; conglutination thrombi
77	360	1.0 c.c. (subcutaneously)	0.003 c.c.	Moderate dyspnea	Recovery 84	Marked distention of lungs; pulmonary hemorrhages and congestion; marked cardiac dilatation; abdominal congestion	Marked congestion; slight distention
8	372	2 c.c. of 0.5 per cent.	0.027 mg.	Kephalin (fresh, sheep; 0.1 per cent., 1 per cent. and 0.5 per cent.) Depression marked	Recovery 60	Lungs collapsed; otherwise normal	Moderate distention, slight congestion, slight hemorrhage

63	226	3 c.c. of 0.1 per cent.	0.014 mg.	None	Recovery	34	Lungs somewhat distended and congested, pulmonary hemorrhages, slight cardiac dilatation, moderate abdominal congestion	Marked distention, marked congestion, slight hemorrhage
12	227	3 c.c. of 0.1 per cent.	0.014 mg.	None	Recovery	42	Lungs collapsed, slight cardiac dilatation	Slight congestion, slight hemorrhage
76	340	3.3 c.c. of 1 per cent.	0.1 mg.	None	Recovery	70	Moderate cardiac dilatation and pulmonary congestion, lungs collapsed	Moderate distention, marked congestion, marked hemorrhage
54	290	3.0 c.c.	0.031 mg.	Dyspnea, tremors, shivering	Trikresol (0.3 per cent. in normal saline)		Marked inflation of lungs; hemorrhages, cardiac dilatation, no abdominal congestion	Marked distention, marked congestion, slight hemorrhage
59	230	3.0 c.c.	0.036 mg.	Tremors; no dyspnea....	Recovery	30	Lungs collapsed and congested, no cardiac dilatation, slight abdominal congestion	Marked congestion
74	320	3.0 c.c.	0.026 mg.	Tremors; mild convulsions; increased respiration	Recovery	38	Lungs collapsed marked pulmonary congestion with some hemorrhages; slight abdominal congestion	Moderate distention, marked congestion, marked hemorrhage
1	240	2 c.c.	0.01 c.c. (0.09 mg. NaCl)	None	Saline controls (0.9 per cent. NaCl)		Lungs collapsed; otherwise normal	
7	350	10 c.c.	0.03 c.c. (0.26 mg. NaCl)	None	Recovery	86	Lungs collapsed; otherwise normal	Slight congestion or normal
46	235	3 c.c.	0.012 c.c. (0.11 mg. NaCl)	None	Recovery	48	Lungs collapsed; otherwise normal	Marked congestion
52	220	3 c.c. (0.85 per cent NaCl)	0.014 c.c. (0.12 mg. NaCl)	None	Recovery	41	Very slight lung inflation and cardiac dilatation	Marked congestion; thrombosis in few large veins

observed and careful necropsies and microscopic study of the lungs were made on all animals after they died or were killed. If the effects were not immediately fatal, the animals were killed by a blow at the end of about half an hour.

TABLE 1.—PRECIPITATION OF PROTEIN BY HEAT AND SALTING *

	Heat	Addition of NaCl After Heating
Hemostatic serum	+ st.	+ st. Flocculent
Thromboplastin (fresh, Squibb).....	+ Cloudiness	+ Fine flocculent precipitate; separates
Thromboplastin (fresh, Armour).....	+ st. Flocculent; separates on standing	+ st. Large flocculent precipitate separates
Kephalin (fresh, sheep) 0.1 per cent.	+ v.sl. Clouding	+ Cloudiness more marked; no floccules separate
Coagulen (dry, fresh) 5 per cent.	‡ (?) v. sl. Cloudiness	+ Cloudiness not marked; no floccules separate
Coagulen solution 3 per cent. (from ampoule)	None or very doubt- ful	Marked cloudi- ness; no particles separate
Kephalin (old, sheep) 0.1 per cent.	None or very doubt- ful	Marked cloudi- ness; no floccules separate
Kephalin (fresh, Armour) 0.1 per cent.	+ v. sl. Clouding	+ st.

* The signs and abbreviations in the table have meanings as follows: (+) precipitate formed; st. = strong; v. sl. = very slight; (?) = doubtful.

The necropsy was conducted immediately after death and blocks of the lungs, usually both lower lobes, cut not more than 4 mm. thick. These were placed in Zenker fluid for twenty-four hours, washed in freely running water for twenty-four hours and placed in 70 per cent. alcohol. Then they were run through graded alcohols, imbedded in paraffin and stained with hematoxylin and eosin.

The volume of solution injected ranged from 0.5 to 3 c.c., and the dosage of the individual agents was varied. In some experiments this was somewhat higher per gram of animal than the therapeutic dosage of the agents. However, the dosage of the thromboplastic agents is very elastic and is frequently advised ad libitum owing to the impression that they are non-toxic. If no effects are demonstrable with maximal doses, none would be expected with small, and the statements made concerning the harmlessness of these agents would be more justified. Therefore, we believe that the use of large doses in these experiments is correct in principle. It should be remembered also that repeated medication with these agents is the rule rather than the exception.

The following summary of doses expressed in milligrams per gram body weight and calculated on the basis of a 60 kilo individual indicates that the dosage recommended or used by others is not seriously different from the higher doses used by us:

Coagulen: 0.083 mg. per gm. intravenously advised in the manufacturer's literature for hemophilia. 1 per cent. and 3 per cent. strengths are recommended.

Kephalin: 0.125 mg. per gm. intravenously, and 0.05 gram subcutaneously produced a rise of temperature (Lucas and Hurwitz);³ 0.1 mg. per gram in dogs intravenously (Howell).⁴

Thromboplastin: 2 c.c. of 10 per cent. solution in rabbits (Hess)⁵; not recommended for use intravenously in human individuals but is used subcutaneously 10 to 20 c.c. every twenty-four hours = 0.00033 c.c. per gram (maximal).

Hemostatic serum: 1 to 2 c.c. repeated every four to six hours subcutaneously or intravenously (manufacturer). The maximal dose in twenty-four hours would be 12 c.c. or about 0.0002 c.c. per gram (0.2 c.c. per kilogram).

Kephalin, thromboplastin and coagulen are recommended intramuscularly by Bastedo⁶ in dangerous hematemeses.

3. Hurwitz and Lucas: Arch. Int. Med. **17**: 543, 1916.

4. Howell: Harvey Lectures, 1916-17, series 12, pp. 273-324.

5. Hess: J. A. M. A. **64**: 1395, 1915.

6. Bastedo: Am. J. Med. Sc., **157**: 99, 1919.

The results that were obtained with both small and large doses are summarized in Table 2.

THROMBOPLASTINS AND HEMOSTATIC SERUM

The following agents which were injected intravenously and subcutaneously into guinea-pigs were found to produce marked anaphylactoid symptoms, the claims of the manufacturers regarding the harmlessness of these agents to the contrary notwithstanding: Thromboplastin (Squibb; fresh), thromboplastin (Armour; fresh) and hemostatic serum. It is probable that the symptoms in part at least were due to the preservative, since all animals injected with equal quantities, that is, 3 c.c. of 0.3 per cent. tricresol in normal saline showed similar symptoms, though not as marked, and the changes at necropsy were also similar in some of the animals. However, tricresol was not fatal, while thromboplastin was. Presumably, therefore, the proteins are responsible. This is confirmed by the fact that the animals injected with kephalin and normal saline were practically unharmed. The data in Table 1 indicate that the thromboplastins and hemostatic serum are rich in proteins, while the kephalins are not.

The changes at necropsy were also more severe than those observed after the injection of saline and tricresol. That is, the thromboplastins and hemostatic serum caused more distention and congestion of the lungs; also hemorrhages and greater cardiac dilatation. There was also more abdominal congestion. The majority of the animals injected with saline showed practically no symptoms and no changes at necropsy, these being somewhat more pronounced after tricresol, but not nearly as marked as after the thromboplastic agents.

Microscopically, the difference between the controls (saline and tricresol) and the thromboplastic agents was even more marked. That is, thromboses in the lungs were quite common with the thromboplastins and hemostatic serum, and entirely absent in the lungs of animals injected with tricresol and only one out of

four animals injected with saline showed few thrombi. The presence of thrombi together with the marked congestion, both abdominal and pulmonary, hemorrhages in the lungs and cardiac dilatation, indicate a serious injury to the circulation.

COAGULEN

Coagulen may be described separately, since it contains no tricrosol and is practically protein-free. However, the changes produced after the intravenous and subcutaneous injections of large and small doses of both the fresh dry preparation and the sterile solution in ampules resemble closely those produced by the agents rich in protein (hemostatic serum and the thromboplastins). The respiratory symptoms were more pronounced than the thromboplastins and hemostatic serum. Dyspnea and lung inflation were more pronounced and there was a greater respiratory rate. The circulatory changes as indicated by hemorrhages, congestion, thrombi and cardiac dilatation were about the same as those with the protein rich agents. The principal difference was the absence of fatalities even with the largest doses used. Nevertheless, the conclusion that coagulen is distinctly injurious, producing definite anaphylactoid symptoms, when injected intravenously, and subcutaneously is justified.

Accordingly, precautions should be exercised in the intravenous and possibly also subcutaneous injection of all these agents, namely, the thromboplastins, hemostatic serum and coagulen. This is contrary to the claims of harmlessness made by the manufacturers.

KEPHALIN

The intravenous injection of kephalin in weak (0.1 per cent.) and high concentrations (0.5 per cent.), using ordinary doses, produced no demonstrable symptoms in guinea-pigs. The blood of these animals appeared to clot in about the same way as their fellows injected with saline and other agents. Only one out of the four animals injected showed postmortem

evidence of injury to the respiratory and circulatory systems. Microscopically, the pathologic changes were also moderate. Kephalin, therefore, is a relatively harmless substance as compared with the other thromboplastic agents studied. Apparently no serious contraindications to its use intravenously exists. When high concentrations are used, the possibility of fat embolism should be borne in mind. Neither emboli nor thrombi, clumping of cells or intravascular clotting were observed in the experiments performed.

In this connection it is interesting to note that Bulger ⁷ regards the change in blood coagulability in anaphylactic shock as due to changes in thromboplastin (kephalin, etc., presumably). If this is true, and since blood anaphylactic shock has been repeatedly observed to possess a prolonged coagulation time, the injection of thromboplastic agents (kephalin, etc.) theoretically should mitigate the difficulty, if not shock itself. However, our results indicate that certain of these agents produced the signs and symptoms of shock and others did not, and the blood of such animals behaved no differently from that of the controls and other animals used with a series of different agents. It is doubted, therefore, if thromboplastin per se (or thromboplastic agents) plays any causal rôle in the diminished coagulability of blood of anaphylactic shock.

CONCLUSIONS

1. The thromboplastic agents rich in protein (thromboplastin [Squibb and Armour] and hemostatic serum) are distinctly harmful when injected intravenously and subcutaneously in guinea-pigs, producing anaphylactoid symptoms and injury to the circulation; and death with large doses of thromboplastin intravenously.

2. Coagulen, which contains only traces of native protein, produced more pronounced anaphylactoid symptoms and also injured the circulation.

7. Bulger: *J. Inf. Dis.*, **23**: 522, 1918.

3. The injurious effect of the thromboplastins⁹ and hemostatic serum may be accounted for in part by tricresol (the preservative) and to a greater extent by the protein fraction. This does not appear to be true of coagulen which contains neither tricresol nor native protein.

4. Caution is therefore necessary when employing these agents intravenously and subcutaneously.

5. Kephalin appears to be relatively harmless as compared with the other thromboplastin agents studied.

8. The Thromboplastin (Squibb) was the ordinary variety, and not that marketed as "Thromboplastin Hypodermic" (Squibb). Since this report was written, we have been informed that the latter is heated. We, therefore, can not assert whether the "hypodermic" produces the same effects as the ordinary thromboplastin.

A CLINICAL STUDY ON THE USE OF CALOMEL INUNCTIONS *

H. N. COLE, M.D.

AND

SIDNEY LITTMAN, M.D.

CLEVELAND

(From *The Journal A. M. A.*, Nov. 8, 1919, p. 1409)

Since the earliest times, mercury inunction has been used for the treatment of syphilis. The drug, however, has always been employed in the form of an ointment of the metallic mercury. Recently Wile and Elliott¹ and Schamberg, Kolmer, Raiziss and Gavron² have suggested the use of an ointment made of calomel; this, for several reasons: The blue ointment is very uncleanly; it leads to discovery, and it frequently sets up an irritation of the skin. As calomel is more cleanly, and as the latter four authors felt that it is fully as well absorbed through the skin as the ordinary blue ointment, they suggested that it be used for inunction purposes in a formula consisting of:

	Gm.	
Hydrargyri chloridi mitis.....	3	gr. xlv
Lanolini	1	gr. xv
Adipis benzoinati	2	gr. xxx

The argument of these writers seemed convincing, and we commenced using calomel inunctions, especially in private practice, in the spring of 1917. Since then the use of the calomel rubs has become so extensive that a manufacturing drug house has brought out an

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* From the Department of Dermatology and Syphilis of the Western Reserve University School of Medicine and of the Cleveland City Hospital.

1. Wile, U. J., and Elliott, J. A.: Mode of Absorption of Mercury in the Inunction Treatment of Syphilis, *J. A. M. A.* **68**: 1024 (April 7) 1917.

2. Schamberg, J. F.; Kolmer, J. A.; Raiziss, G. W., and Gavron, J. L.: Experimental Studies of the Mode of Absorption of Mercury When Applied by Inunction, *J. A. M. A.* **70**: 142 (Jan. 19) 1918.

ointment of calomel which is a little more pleasant in form than the ordinary preparation, though probably no more efficient.

It was only after we had used this for some time that we began to feel more and more that it was not doing what was required of it. In the first place, we noticed, as was later called to our attention by Dr. Torald Sollmann of the Pharmacology Department of the Western Reserve University, that the patient did not get salivated. Moreover, it seemed that many times the patients did not react to treatment as they had formerly, when we were using inunctions of blue ointment instead of calomel, or when using injections of mercury. Therefore, in order to test out the efficacy of the inunction of calomel thoroughly, we commenced a series of experiments, using the fresh, clinical syphilis in our wards in the venereal disease department of the Cleveland City Hospital. We shall report the results of tests carried out in the past year or so in fifty-four cases. This investigation was undertaken at the suggestion of the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

Before describing these individual cases, we would say that we have tried to be as unprejudiced as is possible. The cases have been carefully followed; routine Wassermann tests on the blood, *Spirochaeta pallida* examinations and spinal fluid examinations were made in every instance. Moreover, in using the ointments, the patients have always been under the observation of an orderly or of a nurse to see that the inunction was carried out through a space of at least thirty minutes for six nights in each week, with a hot bath on the seventh night, and without other medication. Each patient has used an ordinary potassium chlorate mouth wash, but otherwise the run of teeth has been simply that of the teeth that one finds in any free hospital practice, which is to say, usually very poor.

In nearly all cases we used the 50 per cent. calomel ointment described above and made up by the hospital pharmacist. In the few cases which are noted in the clinical histories we employed a proprietary calomel preparation.

It has often been difficult to keep the patient under the calomel rubs for as long a time as we would desire, especially if the patient did not seem to improve in his own estimation. Again, we have found that frequently the patients would get together and talk over the therapy and advise each other as to their estimation of the therapy being used. To overcome this, and to make the experiments still more objective, we finally gave the patients saline injections into the arm during the course of the calomel rubs, so that they would not know that they were not receiving arsphenamin injections. In this manner we did our best to carry on the inunction treatment as long as possible, with practically every patient.

REPORT OF CASES

CASE 1.—G. M., a woman, aged 19, had marked mucous patches on the tonsil and the pillar, condylomas of the vulva and anus and a mixed macular papulosquamous eruption. After thirty-two rubs, the patches were still present on the tonsils, though the skin showed some improvement. Rapid improvement followed the use of arsphenamin at this time.

CASE 2.—A. A., a woman, aged 25, had marked condyloma of the anus and a generalized macular eruption. There was no change after eighteen rubs, and the patient demanded arsphenamin.

CASE 3.—G. H., a man, aged 40, had marked patches on the tonsils, an annular syphilid on the forehead and extensive condylomas. There was no change after eighteen rubs, and because of the patient's insistence he was given treatment with arsphenamin, especially as the spinal fluid cell count had increased from 3 to 50. The patient had very bad pyorrhea, yet showed no gingivitis after thirty rubs.

CASE 4.—E. F., a man, aged 28, had mucous patches on the left pillar and a generalized macular eruption. After eighteen rubs the patches were still present on the pillar, and there was no change in the eruption. Rapid improvement followed treatment with arsphenamin.

CASE 5.—G. L., a woman, aged 21, complaining of headache and rheumatism, had a spinal fluid cell count of 25 and patches on the right soft palate. After twenty-four rubs the patches were much worse in the throat, and the patient was given arsphenamin therapy. Rapid improvement followed.

CASE 6.—W. G., a man, aged 31, had a "veneer" chancre the size of a dime, of three weeks' duration, on the foreskin. During the course of twenty-two rubs, a secondary eruption developed, and large patches appeared on the left tonsil. Rapid improvement followed treatment with arsphenamin.

CASE 7.—N. C., a man, aged 27, had a follicular syphilid. After twenty rubs, patches appeared on the left tonsil. He rapidly improved after treatment with arsphenamin.

CASE 8.—N. W., a woman, aged 20, colored, had an extensive annular syphilid over the entire body. The condition became worse after eighteen rubs, and a deep, syphilitic ulcer began on the right tonsil after twenty rubs. Rapid improvement resulted from treatment with arsphenamin. The rubs were continued up to forty with no evidence of stomatitis or gingivitis.

CASE 9.—J. S., a man, aged 28, had a symmetrical papulosquamous syphilid. There was no change after thirty-one rubs. A rapid improvement followed treatment with arsphenamin.

CASE 10.—D. A., a man, aged 45, had a large gumma in the right inguinal region. A history of syphilis was denied. The spinal fluid count was 108 cells. After thirty rubs the gummatous mass showed no change, but the spinal fluid count dropped to 35 cells. We continued the calomel rubs to forty-three with no stomatitis or gingivitis. The gumma healed quickly on one injection of arsphenamin, showing improvement almost within twenty-four hours.

CASE 11.—E. R., a woman, aged 52, had papulocrustaceous and serpiginous lesions on the right arm. They all disappeared after twenty rubs with calomel. She received thirty rubs with no effect on the teeth.

CASE 12.—E. B., a man, aged 18, had a papulosquamous syphilid and areas of lichen syphiliticus. After twenty-five rubs, the eruption on the body was fairly well cleared up, but patches in the throat and on the lips were much more severe. Rapid improvement followed treatment with arsphenamin.

CASE 13.—J. B., a man, aged 29, had marked patches on the tonsils, and macular syphilis. After nineteen rubs the

patches entirely covered the tonsils, though the skin was clear. There was a rapid improvement under arsphenamin treatment. No stomatitis was present after twenty-three rubs.

CASE 14.—A. K., a girl, aged 16, had a chancre on the upper lid and a macular eruption and patches in the throat. There was no change after twelve rubs, and the patient demanded a different treatment.

CASE 15.—J. N., a woman, aged 20, had condylomas and also patches on the left anterior pillar, uvula and soft palate. There was no change after twelve rubs, when the patient escaped from the hospital.

CASE 16.—W. E., a girl, aged 16, had condylomas of the vulva and patches on the left tonsil. After twenty-one rubs the tonsils were still covered with patches when the patient escaped from the hospital.

CASE 17.—J. H. a man, aged 25 had patches in the mouth and the throat, condylomas of the anus, and fissures at the corners of the mouth. After nineteen rubs the oral fissures became condylomatous, with no evidence of gingivitis. There was a rapid improvement after arsphenamin treatment.

CASE 18.—T. S., a man, aged 28, had mucous patches on both tonsils, the soft palate, and inside of the left cheek; and a faint, macular syphilis. After twenty-four rubs the patches were worse, though there was no gingivitis in the mouth. The patient complained severely of rheumatic pains, and of the joints. There was a rapid improvement under treatment with arsphenamin.

CASE 19.—C. B., a man, aged 25, had a faint macular eruption and primary lesion in the balanopreputial sulcus. After eighteen rubs the primary lesion showed no change, and the patient had very severe rheumatic pains. The eruption became more marked. There followed a rapid improvement under arsphenamin treatment, with the teeth showing no gingivitis after twenty-five rubs.

CASE 20.—J. S., a man, aged 42, had a large ulcerocrustaceous syphilid covering the entire abdominal wall. There was a moderate improvement after forty-one rubs, with a miraculous change within forty-eight hours after arsphenamin was given. The teeth showed no gingivitis.

CASE 21.—W. W., a man aged 21, colored, had patches on both tonsils and an annular syphilid on the neck, right elbow and buttock. After thirty rubs the lesions in the throat showed no change, and several circinate lesions had appeared on the right elbow. The patient escaped from the hospital at this point.

CASE 22.—A. W., a woman, aged 29, colored, had an annular syphilid of the face and lips, and around the nose. After eight rubs she developed a marked edema of the skin, followed by desquamation and tenderness. A faint trace of albumin and a few casts appeared in the urine. The condition was diagnosed as calomel dermatitis, and it all cleared up quickly after the calomel rubs were stopped.

CASE 23.—C. S., a woman, aged 24, had patches on the lower lip and lesions on the labia majora. After twenty-six rubs papulosquamous lesions, syphilitic in character, appeared on the arms and neck. There was no change in the patches. Instantaneous results followed the use of arsphenamin.

CASE 24.—R. H., a man, aged 28, had a macular syphilid on the chest, abdomen and back. After twelve rubs the macular eruption was gone, and the mucous membranes cleared. The patient felt fine after fourteen rubs. After thirty-two rubs an extensive mucous patch began to develop on the right posterior pillar. There was no evidence of gingivitis. The spinal fluid count, on the patient's admission, was 43 cells. After thirty-two rubs there were 38 cells. Bloody fluid was found after the patient had received three injections of arsphenamin. He reacted immediately to treatment with arsphenamin and showed no evidence of hydrargyria after forty-three rubs.

CASE 25.—J. A., a woman, aged 17, had condylomas of the vulva and anus, a faint, macular syphilid, and patches on both tonsils and pillars. After twenty-four rubs the condylomas were markedly improved, but the patches in the throat were much worse, though they had showed some improvement after twelve rubs. The patient was given arsphenamin, and results were rapid. She took thirty-six rubs, with no evidence of stomatitis.

CASE 26.—R. H., a woman, aged 18, had mucous patches on both tonsils; a flat, papular syphilid on the face, chin and neck, and a general macular eruption. After eighteen rubs the lesions had all disappeared. The patient developed marked stomatitis and gingivitis after twenty-one rubs. She was then given arsphenamin.

CASE 27.—H. P., a man, aged 28, had mucous patches on the lips, tongue, tonsils, pillars and uvula, and a faint macular eruption. After eighteen rubs the lesions on the lip and tonsils were practically gone, the skin showing a slight desquamation. The patient was then treated with arsphenamin.

CASE 28.—J. D., a man, aged 18, had mucous patches inside the upper lip and over the tonsils, pillars and uvula. After

twenty-two rubs the patches on the left corner of the mouth had become almost condylomatous in character; those in the throat remained the same. There was a rapid improvement under treatment with arsphenamin. The patient took thirty-eight rubs, with no evidence of stomatitis or gingivitis.

CASE 29.—W. M., a man, aged 24, had maculopapular syphilis and condylomas along the sulcus and around the anus. After thirty-four rubs there was no change in the condition. It cleared up rapidly under treatment with arsphenamin. The patient took fifty-one rubs with no evidence of gingivitis or stomatitis.

CASE 30.—R. M., a man, aged 22, had phimosis with a palpable chancre of four weeks' duration. Under treatment with rubs, a papulosquamous syphilid appeared, and after twenty-three rubs the patient had severe patches in the throat and on the upper lip. After thirty rubs he began to complain of loss of hearing which was explained as an extension of the mucous patches and inflammation up the eustachian tubes. After thirty-six rubs the patient was given arsphenamin and rapid improvement followed. No evidence of stomatitis or gingivitis appeared after forty-two rubs.

CASE 31.—A. D., a woman, aged 33, had mucous patches on the tonsil along the edge of the tongue, flat, papular lesions over the labia and general papular syphilis. The patient immediately salivated after twelve rubs. Patches were still present in the mouth after eighteen rubs, though the lesions on the vulva were somewhat better. She was given arsphenamin at this time because of the teeth.

CASE 32.—J. C., a man, aged 32, had marked phimosis with condylomas in the sulcus back of the glans. Spirochetes were present in the lesions. The patient was much worse after fifteen rubs, and was given treatment with arsphenamin. The lesions showed a marked improvement twenty-four hours later.

CASE 33.—J. L., a woman, aged 26, had mucous patches on both tonsils, the pillars and uvula. After seventeen rubs the lesions in the throat were more extensive, and the patient was given arsphenamin. She took thirty-two of the rubs with no evidence of stomatitis or gingivitis.

CASE 34.—J. C., a man, aged 51, had macular syphilis. After ten rubs his eruption was fainter. After twenty rubs definite patches began to appear in both tonsils, and he was given arsphenamin. He had thirty rubs with no evidence of gingivitis. The spinal fluid count, on the patient's admission, was 11 cells. After twenty rubs, there were 22 cells in the spinal fluid.

CASE 35.—J. U., a man, aged 55, had extensive mucous patches on the right anterior pillar, on the shaft of the penis, and on the scrotum. Condylomas of the anus were present, and macular syphilis. After twenty-two rubs, the patches in the throat extended high up on the soft palate, and arsphenamin was ordered. Rapid improvement followed. The patient took thirty-six rubs with no evidence of stomatitis or gingivitis.

CASE 36.—J. R., a man, aged 29, had a macular eruption, extensive mucous patches on both tonsils and on the pillars, and large patches on the under surface of the right side of the tongue. After twenty-one rubs of the proprietary, the lesions on the tongue had become an ulcer, and the patient developed severe headache. He was given arsphenamin, and all symptoms cleared up rapidly. He received twenty-six rubs with no evidence of stomatitis or gingivitis. On the patient's admission, the spinal fluid cell count was 1. After twenty-one rubs, the fluid was bloody.

CASE 37.—J. J., a woman, aged 25, had a fine miliary syphilid. After twenty-four rubs, the rash was fading. After thirty-seven rubs, a follicular syphilid appeared on the face and forehead, and a definite mucous patch on the tonsil. The patient was given arsphenamin, and there was a rapid improvement. She took fifty rubs with no evidence of gingivitis or stomatitis. The spinal fluid cell count, on her admission, was 3. After thirty-seven rubs, there were 27 cells in the spinal fluid. The count dropped to 3 after four injections of arsphenamin. In this case the proprietary preparation was not used.

CASE 38.—L. G., a man, aged 45, had an extensive ulcerocrustaceous syphilid on both legs. After twenty-three rubs of the proprietary preparation, the lesions on the thigh had improved 50 per cent., and the patient had a slight gingivitis in conjunction with an old, extensive pyorrhea. At this time he developed gangrene on his leg. Operation was refused and the patient died.

CASE 30.—Peter H., a man, aged 26, had a generalized maculopapular eruption, with patches in the mouth and on the genitalia. After ten rubs with the calomel, the lesions on the genitalia had disappeared, and the eruption was entirely gone after the patient had taken eighteen rubs. The tonsils were still large and swollen, however, and a new mucous patch appeared on the right tonsil. There was no salivation. The patient was given the diarsenol brand of arsphenamin.

CASE 40.—M. S., a woman, aged 32, had a chancre of the lip, a generalized maculopapular and pustular eruption, iritis of the right eye, patches on the soft palate, and enlarged and reddened tonsils. After ten rubs, there was a slight fading of the eruption. After eighteen rubs, the eruption of the face was clearing up, and there was some fading of the eruption on the body. After thirty-five rubs of the proprietary, the patient developed some new lesions on the right side of the lower lip. There was no improvement in the eye condition until she was given arsphenamin and mercury injections.

CASE 41.—M. P., a woman, aged 26, had a chancre of the lip and mucous patches inside of the left cheek and over the soft palate. After twelve rubs, the lesions cleared up. This patient was not observed further, but was given arsphenamin at once.

CASE 42.—M. M., a woman, aged 32, had a primary lesion of the left labium majus, with marked edema of the parts, and headache and rheumatism. There was practically no reaction to thirty rubs with calomel ointment, but a rapid improvement under arsphenamin treatment.

CASE 43.—C. G., a man, aged 28, had extensive patches in the mouth and over the scrotum. The spinal fluid count was 20 cells. After nineteen rubs, the lesions in the throat and over the scrotum were practically gone. The patient had a slight salivation. He was given the diarsenol brand of arsphenamin and discharged.

CASE 44.—J. F., a man, aged 28, had a history of chancre seven months before. There were no physical signs of syphilis except headache and a ++++ Wassermann reaction. After seven rubs with calomel, the patient was severely salivated.

CASE 45.—J. P., a man, aged 22, had a lesion of the glans of two weeks' duration. Spirochetes were present in large numbers with dark field illumination. The lesion was unchanged after twenty-three rubs, and the patient was given arsphenamin.

CASE 46.—Grace M., a woman, aged 21, had a maculopapular eruption and extensive patches in the mouth. After seventeen rubs the eruption had practically disappeared, but the mucous patches in the throat showed no change, and the patient complained of dizziness and severe headaches. She refused a lumbar puncture. Rubs of 50 per cent. unguentum hydrargyri were given and, after ten of them, all symptoms and lesions disappeared. She was then given arsphenamin and discharged.

CASE 47.—R. C., a woman, aged 21, colored, had condylomas of the vulva and an annular syphilid of the face and neck. The lesions had practically cleared up after sixteen of the calomel inunctions, but, beginning with the twenty-third daily rub, some mucous patches appeared in the throat which lasted until she had had forty-one rubs. The patient then received the diarsenol brand of arsphenamin, and the patches cleared up rapidly. There was no salivation.

CASE 48.—N. B., a woman, aged 23, had a diffuse maculopapular eruption which entirely disappeared after twenty-three rubs. After thirty-two of them, a heavy trace of albumin and casts appeared in the urine. The skin was entirely clear.

CASE 49.—A. G., a woman, aged 24, had a generalized maculopapular eruption, and patches in the mouth. After twenty-three rubs, the mucous membranes and skin were clear, and the treatment was continued until thirty-two rubs had been given. There were no further symptoms.

CASE 50.—L. W., a man, aged 28, had a lesion of the glans of two weeks' duration. There was no reaction to twenty-eight of the calomel inunctions. The lesion cleared up quickly under treatment with arsphenamin. A Wassermann reaction was ++++.

CASE 51.—S. S., a man, aged 31, had a lesion of the glans and prepuce of three weeks' duration, occurring four weeks after intercourse. Spirochetes were present in large numbers. The patient took thirty-eight rubs of calomel with no change in the lesion. It cleared up immediately after the use of arsphenamin.

CASE 52.—M. S., a man, aged 23, had a marked papulopustular syphilid on the head, face, neck, trunk and arms, and patches on the left tonsil. After thirteen rubs with calomel ointment, the patient demanded arsphenamin. All of the lesions reacted quickly.

CASE 53.—N. N., a man, aged 36, had a periurethral chancre of two weeks' duration, and spirochetes were present in large numbers. After ten rubs with 50 per cent. calomel ointment, the patient showed no improvement and demanded arsphenamin. The lesion healed at once.

CASE 54.—F. N., a man, aged 33, had a generalized maculopapular eruption, with a patch on the left tonsil and a mucous patch on the genitalia. There was no change after thirteen rubs with 50 per cent. calomel ointment, and the patient refused further therapy and was given arsphenamin.

ANALYSIS OF RESULTS

An analysis of the foregoing results shows several things: In the first place, we note among these fifty-four cases that nineteen times (35 per cent.) the lesions, especially those on the mucous membranes, became much worse; five times (9 per cent.) they grew worse; sixteen times (29.5 per cent.) there was no change; five times (9 per cent.) there was a slight improvement; three times (5 per cent.) they were quite markedly improved, and three times (5 per cent.) the patients' symptoms disappeared entirely. In other words, there is a great preponderance of instances in which the patients got much worse under therapy, and this despite the fact that over forty rubs to a patient were used eight times (15 per cent.), that twelve times (22 per cent.) between thirty and forty rubs were used, and that thirteen times (24 per cent.) between twenty and thirty were used.

We had hoped to keep all the patients under treatment by calomel inunctions until from forty to fifty rubs had been given. This was impossible, however, as we have explained, for many times the patients refused further therapy, and in many instances they kept getting so much worse that strenuous measures had to be used to check the course of the disease. It will be noted from the reports that there was practically never any improvement in primary lesions when inunctions were used.

It is interesting to note that in one of the cases, after six rubs, a generalized exfoliating dermatitis developed as a result of the calomel inunctions, with a complicated acute nephritis and edema of the entire skin. These lesions all disappeared rapidly after discontinuing the use of the drug.

The lack of salivation is in striking contrast to results after the use of unguentum hydrargyri. It is difficult, if the patient is using unguentum hydrargyri properly, to give him more than eighteen or twenty rubs without marked symptoms of gingivitis and salivation. But in our fifty-four cases in which calomel inunctions were used, this was present in only

three (1.5 per cent.) cases. In one patient there was a slight salivation after eighteen inunctions. In another there was a moderate salivation after nineteen inunctions, and one patient with a bad pyorrhea had a marked salivation after seven inunctions. Salivation was entirely absent in twenty-nine patients (53.5 per cent.) who used from twenty to forty rubs. We wish to call attention particularly to this point, and we agree with Dr. Sollmann that this striking lack of salivation after the use of calomel inunction means a very low grade of mercury absorption.

We are quite sure that if any one will carefully follow a series of cases such as our own, checking them up carefully from day to day, he will come to the same conclusions. Calomel inunctions, though cleanly and pleasant for the patient, do not compare in efficacy with inunctions of unguentum hydrargyri.

CONCLUSIONS

Observations on a series of fifty-four patients treated intensively with calomel rubs show that:

1. Calomel inunctions are almost totally inefficient against primary and secondary syphilis.
2. Calomel inunctions very rarely produce salivation or gingivitis. This means poor absorption of the mercury and explains this clinical inefficiency.
3. Calomel rubs may occasionally produce a dermatitis.

These results have led us to abandon calomel inunctions, and we would strongly advise against their further use in the treatment of syphilis.

THE PREVENTION OF SIMPLE GOITER IN MAN *

THIRD PAPER

O. P. KIMBALL, M.D., J. M. ROGOFF, M.D.

AND

DAVID MARINE, M.D.

CLEVELAND

(From The Journal A. M. A., Dec. 20, 1919, p. 1873)

This report is based on the reexamination of the girls in the public schools of Akron, Ohio, in grades from the fifth to the twelfth, inclusive, made from Nov. 26 to Dec. 3, 1918—nineteen months after beginning the prophylactic use of iodine. The first report¹—a survey of the incidence of thyroid enlargements (goiter)—was based on the examination made in April, 1917. The second report² gave the results of the examination in November, 1917—seven months after beginning the prophylactic use of iodine.

The same classification of the condition of the thyroid has been used as in previous examinations, namely: normal, slight, moderate and marked enlargements, adenomas and persistent thyroglossal tracts.³ The pupils were further examined for gross manifestations of exophthalmic goiter and myxedema. No obvious case of either of these diseases was detected.

ANALYSIS OF THE RECORDS OF NEW PUPILS

The results of these examinations are given in Table 1.

* From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University.

* Aided by a Grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

1. Marine, David, and Kimball, O. P.: Prevention of Goiter in Man, *J. Lab. & Clin. M.* **3**: 40-43 (Oct.) 1917.

2. Kimball, O. P., and Marine, David: Prevention of Goiter in Man, Second Paper, *Arch. Int. Med.* **22**: 41-44 (July) 1918.

3. Persistence of the thyroid tract is the best evidence of enlargement of the thyroid during fetal life.

For comparison and reference, the figures of the original survey in April, 1917, and of the second examination in November, 1917, are added. The subjects included in this table are all new admissions to the public schools and presumably had not previously received iodine. Owing, however, to the very extensive use of iodine in some form, both by the public and the profession, it is probable that some of these pupils had had iodine for one reason or another, but no attempt was made to detect such cases. Iodine administered in any form markedly affects the thyroid.

The figures in the first line represent the results of the original survey of all girls in grades from the fifth to the twelfth, inclusive. The figures in the second line represent: (1) incoming fifth grade girls; (2) girls entering other grades of the public schools between April and November, 1917, and (3) girls of two

TABLE 1.—ANALYSIS OF THE RECORDS OF NEW PUPILS

Date Examined	Total Examined	Total New Cases	Normal Pupils		Enlargements								Adenomas	
					Slight		Moderate		Marked					
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
April, 1917	3,872	3,872	1,688	43.5	1,931	49.9	246	6.3	7	0.2	39	1.0		
Nov., 1917	4,415	1,772	831	47.0	820	46.2	121	6.8						
Nov., 1918	4,277	1,873	1,037	55.4	779	41.6	53	2.8	4	0.2	6	0.3		

schools that accidentally lost the records of those not taking the prophylactic treatment. The figures in the third line represents only girls in the incoming fifth grade and girls entering other grades.

The progressive increase in the percentage of normal thyroids (43.6, 47 and 55.4 per cent.) for the three periods is due to the preponderance of fifth grade girls in the second and third groups. Fifth grade girls average 10 years of age and are for the most part below the age of the greatly increased incidence of thyroid enlargement.

EFFECT OF PROPHYLACTIC TREATMENT

The same method as outlined in the first paper and modified in the second paper was used, i. e., 2 gm. of sodium iodide were given in 0.2 gm. doses for ten con-

TABLE 2.—RECORDS OF PUPILS TAKING PROPHYLACTIC TREATMENT

Date	Number	Thyroids Remaining Normal		Thyroids Enlarged from Normal to Slightly Enlarged		Slightly Enlarged				Moderately and Markedly Enlarged			
		No.	%	No.	%	Unaltered		Decreased		Unaltered		Decreased	
						No.	%	No.	%	No.	%	No.	%
November, 1917.....	764	283	37.0	0	0.0	287	37.6	141	18.4	34	4.4	17	2.2
November, 1918.....	1,121	469	41.8	0	0.0	254	31.6	218	19.4	29	2.6	51	4.5

TABLE 3.—RECORDS OF PUPILS NOT TAKING PROPHYLACTIC TREATMENT

Date	Number	Thyroids Remaining Normal		Thyroids Enlarged from Normal to Slightly Enlarged		Slightly Enlarged						Moderately and Markedly Enlarged					
						Unaltered		Decreased		Increased		Unaltered		Decreased			
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		
November, 1917.....	7,879	637	33.9	259	13.8	759	40.4	10	0.5	103	5.5	106	5.6	5	0.3		
November, 1918.....	1,283	496	38.7	94	7.3	424	33.1	170	13.2	17	1.3	52	4.1	30	2.3		

secutive school days, repeated each autumn and spring. The results are given in Tables 2 and 3. For reference and comparison, the figures for the November, 1917, examination are added.

The most striking fact brought out is that not a pupil in whom the thyroid was normal at the November, 1917, examination, and who took iodine, showed any thyroid enlargement; while of those not taking iodine, 15.9 per cent. showed definite enlargement. This effect is similar to that noted in last year's examination. As was noted last year a distinct therapeutic effect is again observed in that the glands of 38.1 per cent. of the pupils with slightly enlarged glands decreased following the use of iodine, while of the glands of those listed as not taking iodine 27.8 per cent. showed a decrease in size. This difference is much less than that found last year and suggests that many pupils with slight goiter were taking iodine privately. The same therapeutic effect is also noted in those with moderate and marked enlargements, and again the percentage differences between those taking and those listed as not taking iodine is less than last year's figures.

The main effects of the administration of iodine observed during the second year are similar to those noted during the first year. The danger of iodism or of exophthalmic goiter from the use of such amounts of iodine as were given is shown to be negligible.

SUMMARY

1. Simple goiter in man may be prevented on a large scale.
2. The method used is practical and economical, and can be recommended as a public health measure in goiter districts.
3. Two gm. of sodium iodide given twice yearly, as we have indicated, seems adequate.



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